Transglutaminase Labeling

Instruction Manual

for

Substrate Finder Kit

(L001)

Biotin TGase Protein Q- or K-Labeling Kits

(L101; L201)

PEG TGase Protein Q- or K-Labeling Kits

(L102; L103; L202; L204)

ATTO-Dye™ TGase Protein Q- or K-Labeling Kits

(L104; L105; L106; L107; L108; L204; L205; L206; L207; L1208)

For Research & Development Only

Zedira GmbH Roesslerstrasse 83 D-64293 Darmstadt
Tel.: + 49 6151 66628-0 www.zedira.com contact@zedira.com

Manual revision No: RN5.1

Revision date: December 20, 2021

Zedira GmbH Roesslerstrasse 83 D-64293 Darmstadt

Tel.: + 49 6151 325100 www.zedira.com contact@zedira.com

Contents

1. Introduction

Labeling of proteins using transglutaminase is a smart alternative to chemical protein-labeling procedures. Because of transglutaminase substrate requirements generally a lower number of labels is achieved – compared to chemical labeling – resulting in improved product properties:

- Defined degree of labeling
- Defined label position(s)
- Homogenously labeled protein
- Higher solubility in water
- Minimized amounts of unlabeled protein
- No or reduced impact on bioactivites of labeled proteins

Transglutaminase labeling principle

Transglutaminases are a family of enzymes that catalyse the posttranslational modification of proteins by creating an isopeptide bond within or between polypeptide chains.
In more detail, transglutaminases catalyse the acyl transfer reaction between the γ-carboxyamide group of protein-bound glutamine residues and the ε-amino group of protein-bound lysine or primary amines (Figure 1).

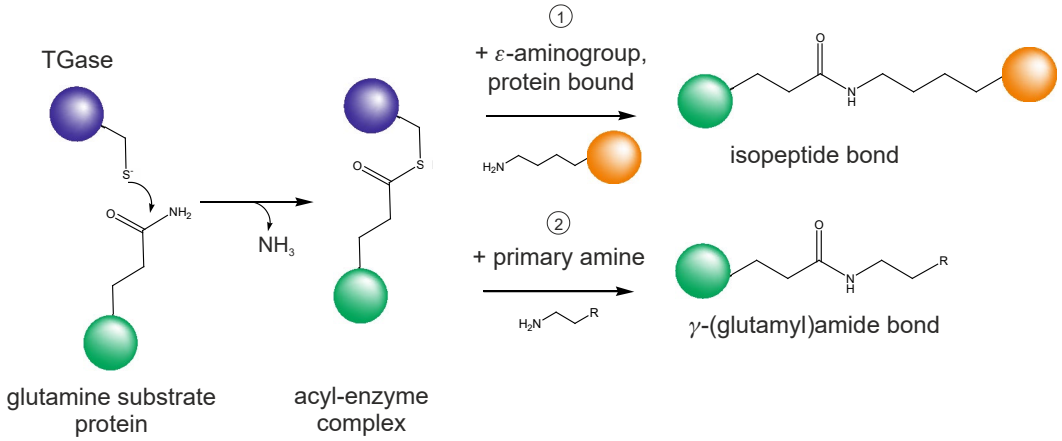


Figure 1: γ(glutamyl)amide bond formation pathways of transglutaminase.

Our labeling kits exploit transglutaminase catalytic activity for **transglutaminase labeling of proteins**. Transglutaminase accepts short glutamine-containing peptides and – as already mentioned – primary amines as substrates. In consequence, protein-bound glutamines can be labeled using primary amines, and protein-bound lysines can be labeled with short glutamine-containing peptides (see Figure 2).
Primary amines as well as glutamine-containing peptides may carry a broad variety of labels like biotin, PEG, fluorescent dyes etc.

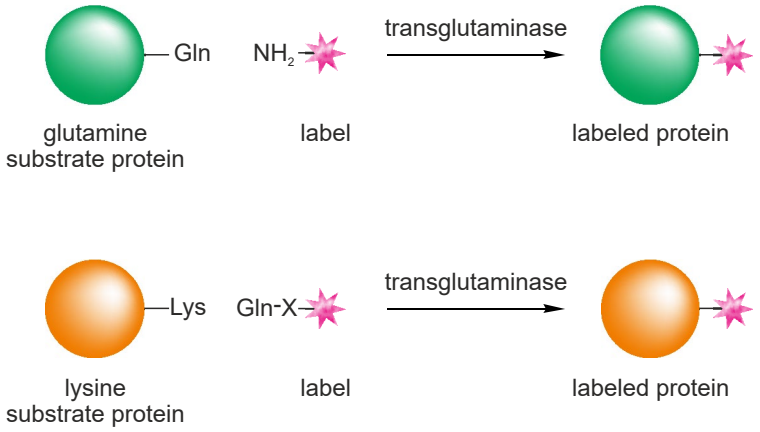


Figure 2: Principle of transglutaminase-catalyzed protein labeling.

The requirements for transglutaminase labeling

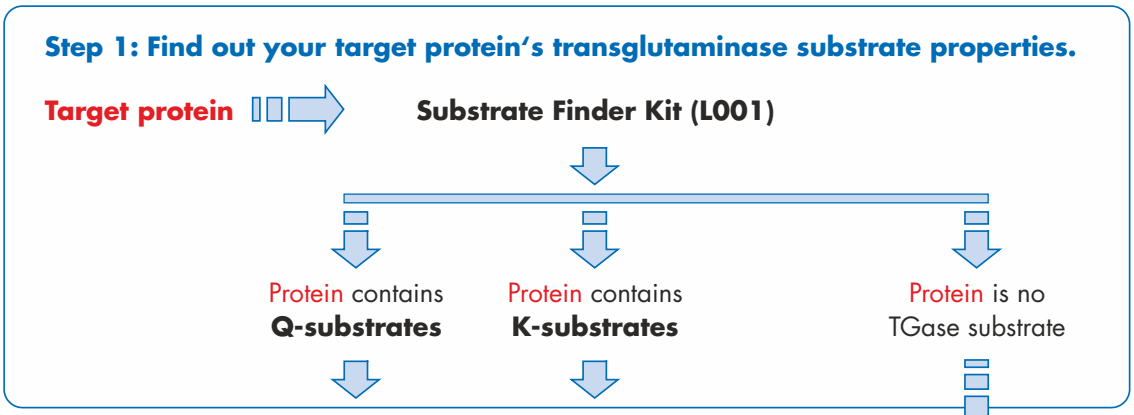
Transglutaminase labeling requires substrate sequences on the target protein surface, which are in general not abundant on proteins.
Therefore, in a first step the target protein has to be analyzed for its transglutaminase substrate properties, which is perfomed using the **Substrate Finder Kit**.
The target protein is accessible to transglutaminase labeling, if it contains either glutamine-substrate sequence(s) or lysine substrate sequence(s) or both.

If the target protein contains neither glutamine- nor lysine-substrate sequences, transglutaminase-substrate tags may be introduced by recombinant techniques.

2. Selection of Labeling Kit

Transglutaminase labeling requires accessible glutamine (Q) or lysine (K) residues on the target protein surface. Our **Substrate Finder Kit** (L001) allows the determination of substrate properties of your target protein. The results obtained by **Substrate Finder Kit** will indicate which **Labeling Kit** is appropriate to introduce the desired label into your target protein, as depicted in Figure 3.

Currently, kits for Glutamine (Q)- or Lysine (K)-labeling with the following labels are available or under development: Biotin, PEG1,088, PEG5,000, ATTO-488™, ATTO-532™, ATTO-550™, ATTO-647N™, ATTO-700™. Please contact us if further labels are required.



Step 2: Label your protein by using the relevant kit.

Label	Q-Labeling Kit	K-Labeling Kit
Biotin	L101	L201
PEG1,088	L102	L202
PEG5,000	L103	L203
ATTO-488™	L104	L204
ATTO-532™	L105	L205
ATTO-550™	L106	L206
ATTO-647N™	L107	L207
ATTO-700™	L108	L208

Figure 3: Substrate Finder Kit based choice of labeling kit.

3. Warnings and Precautions

The labeling kits as indicated on the cover of this manual are applicable only for research and development, not for diagnostic or therapeutic purposes.

Microbial Transglutaminase contains sodium acetate; the MTG-Labeling Buffer contains sodium acetate and sodium azide; Lys-substrate control protein contains 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES); ZQG-CAD-DNS (SK) contains DMSO and hydrochloric acid; Gln-substrate control protein contains tris(hydroxymethyl)aminomethane and sodium chloride; KxD contains sodium acetate.

The above mentioned reagents may be toxic if ingested. Follow routine precautions for handling hazardous chemicals. Avoid all body contact, wear gloves and eye protection. If one of the reagents comes into contact with skin, wash thoroughly with water. Never pipette by mouth.

Sodium-azide may react with lead and copper plumbing to form explosive metal azides. On disposal, flush with a large amount of water to prevent azide build-up.

4. Storage of the Kits

Store kit at 2 – 8 °C. Store component **MTG at -20 °C**.
Do not use after the expiry date stated on the label of the box.

5. Substrate Finder Kit

Substrate Finder Kit (L001) helps determining your target protein's transglutaminase substrate properties. Your target protein may either contain glutamine (Q) substrates, lysine (K) substrates or both. It may also be in-accessible for transglutaminase-labeling.

The **Substrate Finder Kit**-protocol is based on 200 µL of a 1 mg/mL stock solution of your target protein in order to determine both glutamine and lysine substrate properties. If less protein is available the concentration as well as the sample number (allows smaller volumes) may be decreased.
In a first step, your target protein and the substrate are mixed. The labeling reaction is started by the addition of microbial transglutaminase (MTG). At appropriate time points, samples are taken from the reaction mixture and later on loaded onto an SDS-PAGE-gel. Heating of the samples inactivates MTG and stops the labeling reaction.
To analyse the labeling performance, samples are separated by SDS-PAGE. Dansyl labels are detected via UV light. Finally, a coomassie-stain reveals the quality of your SDS-PAGE-gel and the amount of protein in the respective bands / lanes.

Substrate Finder Kit (L001) allows you to prepare 4 reactions with your target protein plus 1 control reaction testing for glutamine substrates labeling (Q-Labeling) as well as 4 reactions with your target protein plus 1 control reaction testing for lysine substrates (K-Labeling).

5.1. Contents of the Kit

a)	MTG	Microbial Transglutaminase	lyophilized powder
b)	LB	MTG-Labeling Buffer (20 mM Tris-HCl, pH7.2, 300 mM NaCl, 0.02% sodium azide)	10 mL
c)	CQ	Q-substrate Control Protein	lyophilized powder
d)	SQ	KxD	lyophilized powder
e)	CK	K-substrate Control Protein	lyophilized powder
f)	SK	ZQG-CAD-DNS	30 µL

5.2. Materials Required but not Supplied

a) 1.5 mL Low Bind reaction tubes (e.g. Eppendorf)

b) Pipettes for 10, 100 and 1000 µL and tips

c) Shaker for 1.5 mL reaction tubes

d) Heat Block (100 °C) for reaction tubes

e) SDS-PAGE gels (e.g. 10%) and device, Coomassie-staining

f) 5x Loading buffer
(e.g. 5x AP: 2% (w/v) SDS, 50% (v/v) Glycerin, 62.5 mM Tris-HCl pH 6.8, 5% (v/v) β-Mercaptoethanol, 0.06% (w/v) Bromophenol Blue)

g) Gel documentation device and UV-table

5.3. Reagent and Sample Preparation

Before opening and using the components, they must have reached ambient temperature (23 ± 3 °C). Do not pool components from different kits, due to possible different handling of the kits. While preparing reagents do not allow foam formation.

Optional: Instead of MTG-Labeling Buffer you may use your own buffer system for reconstituting the reagents and running the labeling reaction. The Substrate Finder Kit is tested for pH-range 6.0 – 7.5. Beyond this range, please assay the control proteins in order to ensure the kit's performance.

a) CQ Reconstitute Q-substrate Control Protein (CQ)
by adding 200 µL MTG-Labeling Buffer (LB) and mix gently.

b) SQ Reconstitute KxD (SQ) in 600 µL MTG-Labeling Buffer (LB)
and mix thoroughly.

c) CK Reconstitute K-substrate Control Protein (CK)
in 230 µL MTG-Labeling Buffer (LB) and mix thoroughly.

d) SK Add 570 µL MTG-Labeling Buffer (LB) to ZQG-CAD-DNS (SK) and mix gently.

e) MTG Reconstitute MTG by adding 170 µL deionized water.

The reconstituted reagents are stable for several hours at 2 – 8 °C. For extended storage freeze reconstituted components at -20 °C.

f) Prepare a 1 mg/mL stock solution of your target protein preferentially in MTG-Labeling Buffer (LB) provided with this kit, or your preferred buffer.

5.4. Procedure

Please protect samples from direct sun light exposure.

5.4.1. Reaction mix Preparation

Determination of **glutamine** substrates (Q-Labeling):

a) **Control protein** master mix: Transfer 100 µL CQ into a 1.5 mL reaction tube, add 100 µL SQ and mix.

b) Your **target protein** master mix: Transfer 100 µL of your target protein (1 mg/mL) into a 1.5 mL reaction tube, add 100 µL SQ and mix.

Determination of **lysine** substrates (K-Labeling):

c) **Control protein** master mix: Transfer 100 µL of CK into a 1.5 mL reaction tube, add 100 µL SK and mix gently.

d) Your **target protein** master mix: Transfer 100 µL of your target protein (1 mg/mL) into a 1.5 mL reaction tube, add 100 µL SK and mix gently.

5.4.2. Assay Procedure

Steps below are **identical** for determination of **glutamine** as well as **lysine** substrates:

e) Preparation of negative control gel sample. Take out 20 µL of each reaction mix (prepared according to a, b, c and d) and add 5 µL 5x Loading buffer (not supplied). Heat for 10 minutes at 100°C (or apply conditions you generally use when analysing your target protein on SDS-PAGE).

f) Start the labeling reaction: add 10 µL MTG to your reaction mix and mix gently.

g) Incubate at room temperature in a shaker (~400 rpm).

h) After 5, 30, and 60 minutes take 20 µL samples from the labeling reaction and add 5 µL 5x loading buffer. Heat **immediately** 10 minutes at 100°C.

i) Load 5 µL of each gel sample onto an 10% SDS-PAGE-gel and run electrophoresis **protected from light**.

j) Place your **unstained** gel onto a UV-table in order to visualize the dansyl-label and document.
UV signal depends on the settings of the UV-table. Please test several settings, use control proteins as indicator for UV-illumination.

k) Stain your gel with Coomassie brilliant blue and document.

5.5. Procedure Flow Chart

Reagent preparation

Labeling of glutamine residues (Q-Labeling)

Add 200 µL of MTG-Labeling Buffer (LB) and mix gently

Add 600 µL of MTG-Labeling Buffer (LB) and mix thoroughly

Labeling of lysine residues (K-Labeling)

Add 230 µL of MTG-Labeling Buffer (LB) and mix gently

Add 570 µL of MTG-Labeling Buffer (LB) and mix thoroughly

MTG

Add 170 µL of deionized water and mix gently

Reaction mix preparation

Labeling of glutamine residues (Q-Labeling)

Transfer 100 µL CQ **or** 100 µL of **your sample** into a reaction tube and add 100 µL SQ and mix

Q-reaction mix

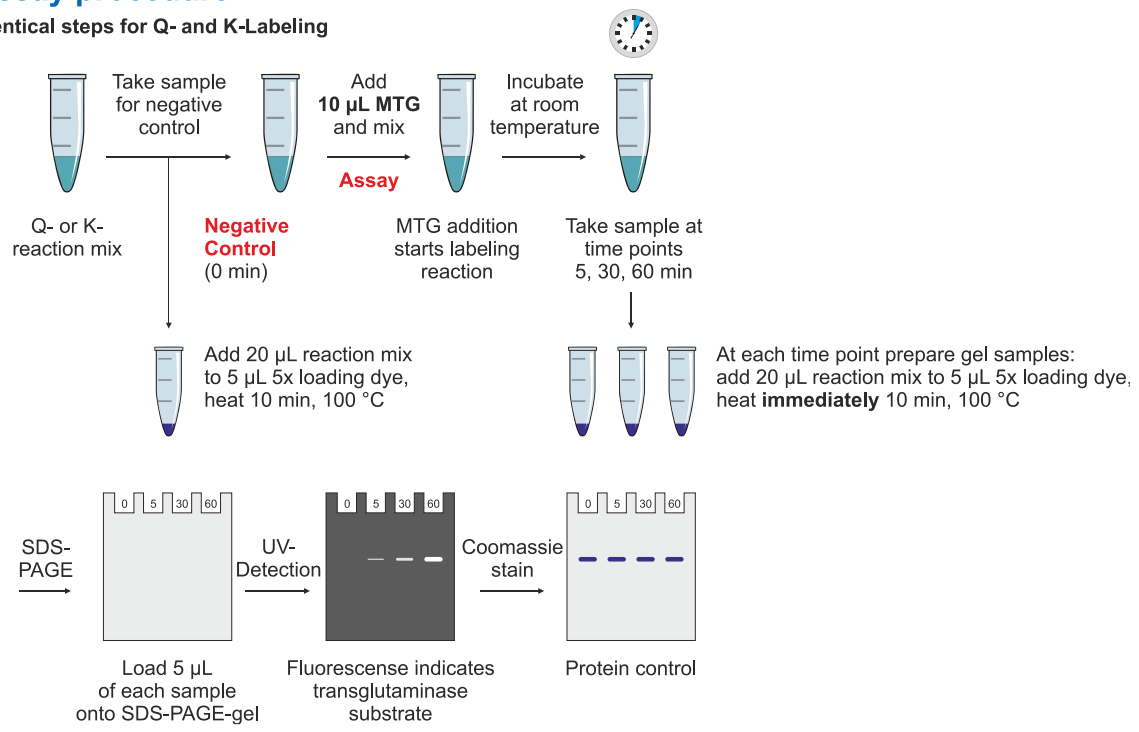
Labeling of lysine residues (K-Labeling)

Transfer 100 µL CK **or** 100 µL of **your sample** into a reaction tube and add 100 µL SK and mix

K-reaction mix

Assay procedure

Identical steps for Q- and K-Labeling



5.6. Interpretation of Results

The analysis of your target protein's substrate properties is performed by combination of UV-illuminated gel and coomassie stained gel.
In Figure 4 results are depicted schematically. Here, the gels on the left side show UV-detection of dansyl-labels, while the gels on the right side show Commassie stained gels. Assuming that equal amounts of target protein were loaded onto the gel, a qualitative interpretation of UV detection is possible.

You can use Figure 4 as guideline for interpreting your own results. Real life examples are given in section 5.7. In the following, the different results you may obtain for your target protein are explained:

Target protein contains only Q-substrate(s)
This situation would result in gels as shown in Figure 4 (i).
Substrate properties are revealed in the UV-illuminated gel (Figure 4 (iA)). The fluorescence intensity of the target protein band increases for SQ (KxD)-samples along the transglutaminase reaction time, while there is no fluorescent band in the SK (Z-QG-dansyl)-samples. The strong fluorescence close to the electrophoretic front is due to the dansyl-substrates (SQ or SK).
The Coomassie-stained gel (Figure 4 (iB)) indicates that identical amounts of target protein. MTG can be seen at 38 kDa in the respective lanes.
Therefore, the target protein contains glutamine substrates and consequently can be labeled with primary amine-based label-conjugated substrates using the following transglutaminase labeling kits: L101 (Biotin), L102 (PEG1,088), L103 (PEG5,000) or L104 (Atto488™).

Target protein contains only K-substrate(s)
This situation would result in gels as shown in Figure 4 (ii). The UV-illuminated gel and Coomassie-stained gel can be interpreted in analogy to (i).
Substrate properties are revealed in the UV-illuminated gel (Figure 4 (iiA)). The fluorescence intensity of the target protein band increases for SK (Z-QG-Dansyl)-samples along the transglutaminase reaction time, while there is no fluorescent band in the SQ (KxD)-samples. The strong fluorescence close to the electrophoretic front is due to the Dansyl-Substrates (SQ or SK).

Thus, the target protein contains lysine substrates and consequently can be labeled with glutamine-containing peptides-based label-conjugated substrates using the following transglutaminase labeling kits: L201 (Biotin), L202 (PEG1,088), L203 (PEG5,000) or L204 (Atto488™).

Protein contains both Q- and K-substrates

If your protein contains both accessible glutamine and lysine residues (Figure 4 (iii)), you can choose either the Q-Labeling Kits or the K-Labeling kits. In this case MTG may cross-link your target protein resulting in dimers or multi- to polymers. The cross-linking reaction can be suppressed by increased labeling-substrate concentrations, reduced labeling time or reduced MTG-concentration.

Protein is no TGase substrate

In case UV-light does not show fluorescent bands, your target protein is no MTG substrate (Figure 4 (iv)).

However, it is an option to add a transglutaminase substrate sequence tag to your protein by recombinant techniques in order to render your protein accessible to transglutaminase labeling.

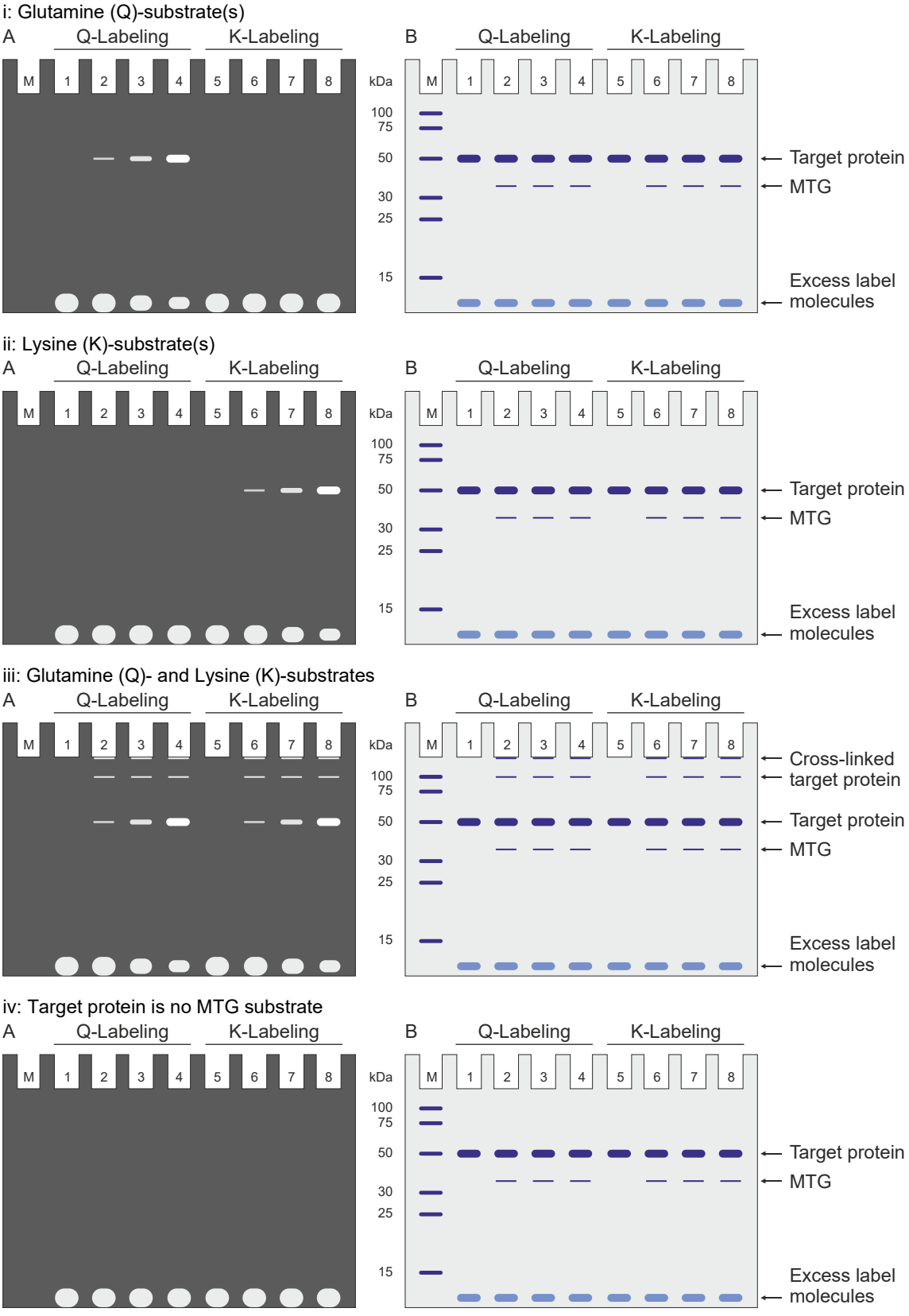


Figure 4: Schematically depicted results of Substrate Finder Kit. A = UV-illuminated gel; B = Coomassie stained gel; M = Protrein Marker; 1-4 = Labeling of free glutamine residues with SQ (timepoints 0 min, 5 min, 30 min, 60 min; 5-8 = Labeling of free lysine residues with SK, timepoints respectively.

5.7. Examples

In the following sections some examples for **Substrate Finder Kit** results are presented. Sample preparation and assay were performed according to the description in sections 5.3 and 5.4.

5.7.1. Protein contains only Q-substrates

N,N-Dimethylcasein (DMC) is a bovine casein-derivative where ε-amino-groups of lysine are di-methylated. Thus no lysine-residues are available for transglutaminase-reaction anymore.
In the Q-labeling lanes (Figure 5A, lanes 2-4) a dominant band and several minor bands are visible. N,N-Dimethylcasein thus contains Q-substrates and can be labeled with the Q-labeling kit series (L101 (Biotin), L102 (PEG1,088), L103 (PEG5,000) or L104 (Atto488™)).
The UV-illuminated gel and the Coomassie-stained gel (Figure 5B, lanes 1-4) further show that the N,N-Dimethylcasein-preparation contains several proteins which are Q-labeling substrates. Further, the migration-behavior of proteins changes with increased labeling time, which may be the result of a high number of labels per N,N-Dimethylcasein-molecule.
In addition, N,N-Dimethylcasein is a very good MTG-substrate, as the labeling is mostly completed after 5 min labeling time only.
In the K-labeling lanes of the UV-illuminated gel (Figure 6A, lanes 2-4), only substrate-bands are visible, indicating that no K-substrates are available.
Finally the coomassie stained gel (Figure 5B and Figure 6B, lanes 1-4) confirms that equal amounts of protein were loaded onto the gel.

The control proteins CQ and CK are labelled in the respective reactions (Figure 5 and Figure 6, lands 5-8). However, the UV-illuminateion reveals a weaker signal. The control proteins contain only one or two residues (Q or K) accessible to MTG labeling. In contrast, DMC contains several accessible Q-residues, explaining the illumination difference observable in Figure 5.
Assaying the control proteins helps to determine the kit's performance, especially if changes in parameters, e.g. buffer conditions, are considered.

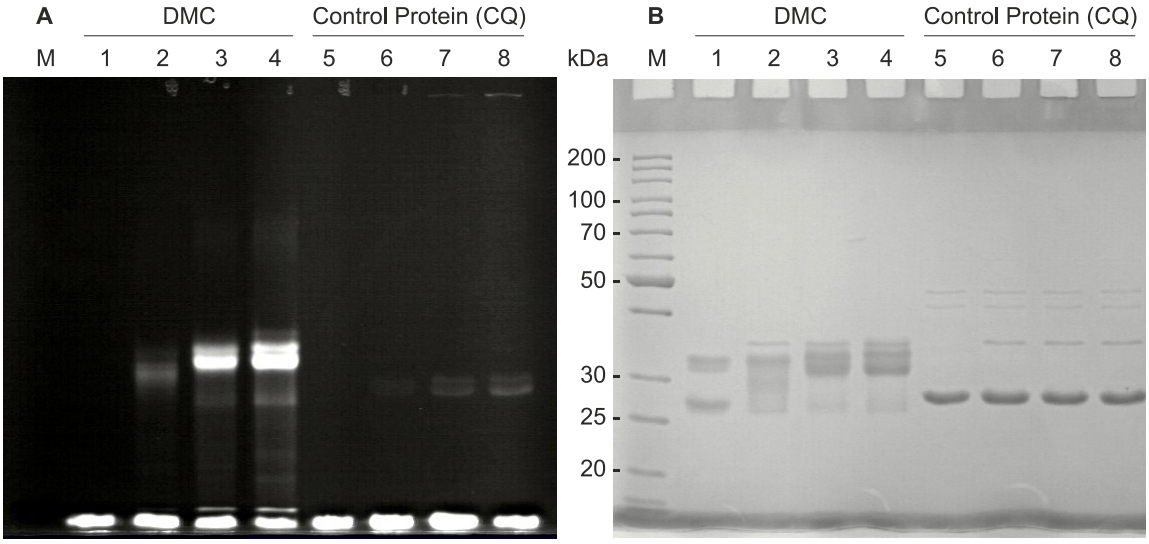


Figure 5: **Q-Labeling** of N,N-Dimethylcasein. A) UV-illumination of dansyl-residues and B) Coomassie stained SDS-PAGE-gel. M = Protein Marker; 1-4 = Target protein DMC, samples taken at 0 min, 5 min, 30 min and 60 min; 5-8 = Control protein CQ, samples taken at 0 min, 5 min, 30 min and 60 min.

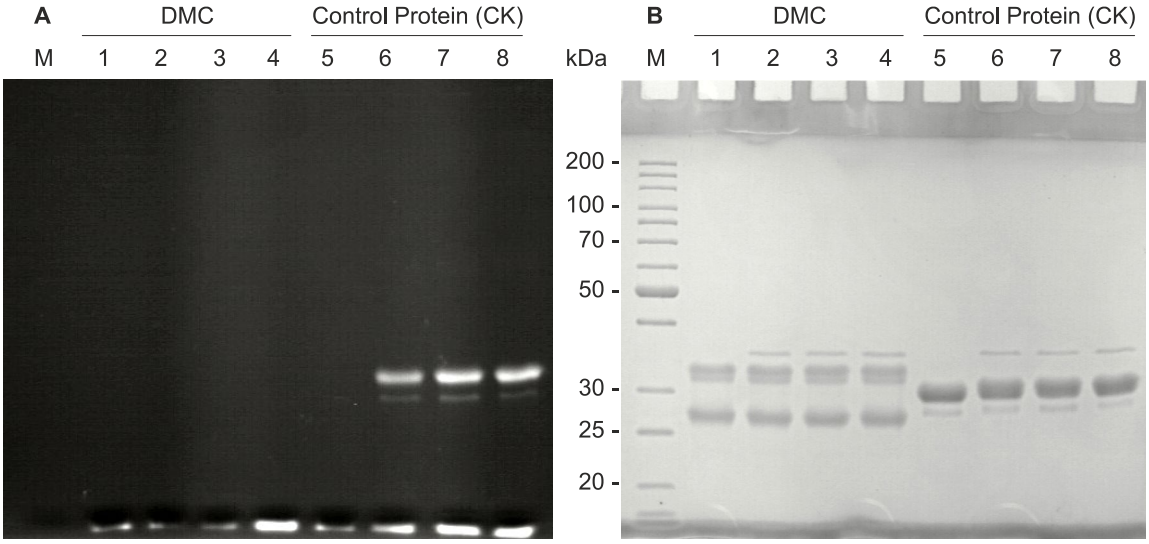


Figure 6: **K-Labeling** of N,N-Dimethylcasein. A) UV-illumination of dansyl-residues and B) Coomassie stained SDS-PAGE-gel. M = Protein Marker; 1-4 = Target protein DMC, samples taken at 0 min, 5 min, 30 min and 60 min; 5-8 = Control protein CK, samples taken at 0 min, 5 min, 30 min and 60 min.

5.7.2. Protein contains both Q- and K-substrates

Assaying bovine α-casein with Substrate Finder Kit (L001) revealed that α-casein contains Q- and K-substrates (Figure 7). However, K-labeling yields better results (more intensive fluorescense in lane 6-8 compared to lane 2-4, Figure 7A).

Not only the Dansyl-Cadaverine label is incorporated into α-casein, but also α-casein itself is cross-linked, shown by multimers and protein aggregates in the gel pockets. Excessive ammount of label molecules is visible at the bottom of the gel.

The Coomassie stained gel (Figure 7B) confirms that equal amounts of protein were loaded onto the gel.

In this example no control proteins were assayed.

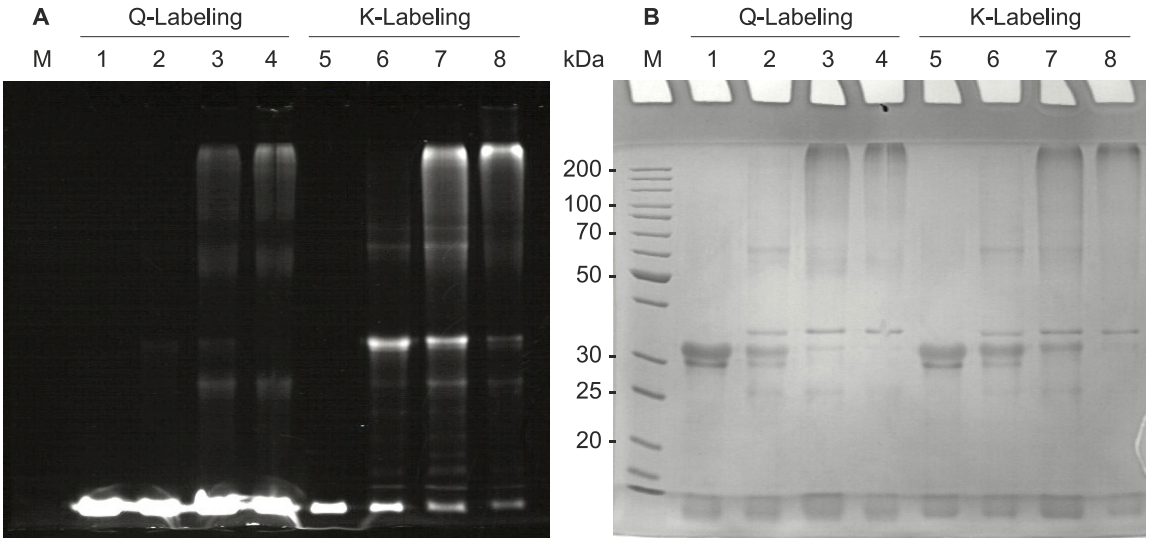


Figure 7: Labeling of α-casein. A) UV-illumination of dansyl-residues and B) Coomassie stained SDS-PAGE-gel. M = Protein Marker; 1-4 = Q-Labeling, samples taken at 0 min, 5 min, 30 min and 60 min; 5-8 = K-Labeling, samples taken at 0 min, 5 min, 30 min and 60 min.

5.7.3. Protein contains neither Q- nor K-substrates

Bovine Serum Albumin (BSA) was assayed with Substrate Finder Kit (L001). Samples were taken after 0 min (negative control), 5, 30 and 60 min.
The UV-illumination (Figure 8A) reveals fluorescence only close to the running front of the gel, but no glowing protein bands, indicating that BSA is no MTG substrate.
In this example no control proteins were assayed.

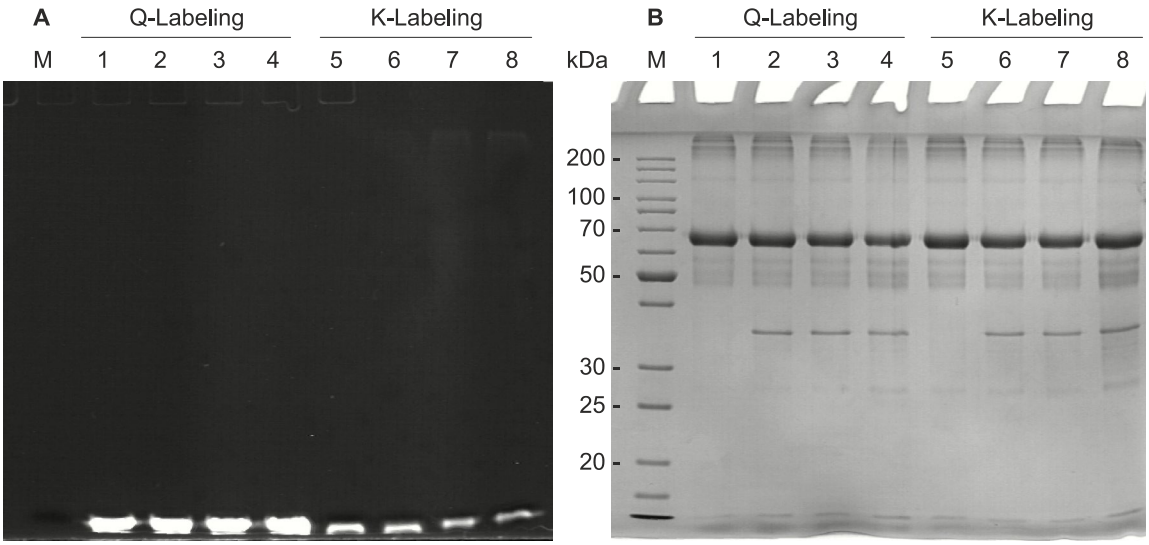


Figure 8: Labeling of Bovine Serum Albumin. A) UV-illumination of dansyl-residures and B) Coomassie stained SDS-PAGE-gel. M = Protein Marker; 1-4 = Q-Labeling, samples taken at 0 min, 5 min, 30 min and 60 min; 5-8 = Q-Labeling, samples taken at 0 min, 5 min, 30 min and 60 min.

6. Biotin TGase Protein Q- and K-Labeling Kits

Biotin TGase Protein **Q**-Labeling Kit (L101) and Biotin TGase Protein **K**-Labeling Kit (L201) are intended for MTG mediated labeling of proteins (> 5 kDa) with biotin. The reagents in both kits are sufficient for biotinylation of 5 x 1 mg protein.

Biotin TGase Protein **Q**-Labeling Kit (L101) is suitable for labeling of proteins containg accessible glutamine (Q) residues. Please follow the instructions indicated with **Q**.
Biotin TGase Protein **K**-Labeling Kit (L201) is suitable for labeling of proteins containig accessible lysine (K) residues. Please follow the instructions indicated with **K**.

Please use the Substrate Finder Kit (L001) initially to determine the suitable biotinylation kit.

The biotinylation protocol is based on 900 µL of your target protein (up to 1.1 mg/mL).
In the first step, your target protein and biotin substrate are mixed. The labeling reaction is started by addition of microbial transglutaminase (MTG). After 30 min, the reaction is stopped by addition of MTG-inhibitor (Stop). **Labeling time may be increased up to 24 h for low reactive proteins.**
To separate your biotinylated protein from excess labeling material, a buffer exchange is performed using a desalting column (GPC). The column has a size exclusion limit of 5 kDa. This step provides the opportunity for a quick and easy buffer exchange into a buffer best suitable for your downstream application.
Labeling performace should be analyzed by SDS-PAGE and Western Blot with consecutive detection of Biotin, e.g. using streptavidin-AP-conjugate.

6.1. Content of the Kits

a)	MTG	Microbial Transglutaminase	lyophilized solid	1 vial
b)	LB	MTG-Labeling Buffer (20 mM Tris-HCl, pH7.2, 300 mM NaCl, 0.02% sodium azide)	100 mL	1 bottle
c)	Stop	MTG-Blocker	lyophilized solid	5 vials
d)	S**Q**-Bio	Biotinyl Substrate for Q-Labeling	white powder	5 vials in kit L101
	S**K**-Bio	Biotinyl Substrate for K-Labeling	20 µL	5 vials in kit L201
e)	GPC	Purification column	--	5 columns

6.2. Materials Required but not Supplied

a) 1.5 mL Low Bind reaction tubes (e.g. Eppendorf)

b) Pipettes for 10, 100 and 1000 µL and tips

c) Shaker for 1.5 mL reaction tubes

d) Rack for purification columns

e) Waste beaker

6.3. Reagent and Sample Preparation

Before opening and using the components, they must have reached ambient temperature (23 ± 3 °C). Do not pool components from different kits, due to possible different handling of the kits. While preparing reagents do not allow foam formation.

Optional: Instead of MTG-Labeling Buffer you may use your own buffer system for reconstituting the reagents and running the labeling reaction. The Biotinylation Kits are tested for pH-range 6.0 – 7.5. Beyond this range, please assay the control proteins of Substrate Finder Kit (L001) in order to ensure the kit's performance.

a) **Q** Reconstitute Biotinyl substrate for Q-Labeling (SQ-Bio) by adding 180 µL MTG-Labeling Buffer (LB) and mix gently (Kit L101).

K Add 180 µL MTG-Labeling Buffer (LB) to Biotinyl substrate for K-Labeling (SK-Bio) and mix gently (Kit L201).

b) Stop Add 60 µL MTG-Labeling Buffer (LB) to MTG-Blocker and mix gently.

c) MTG Reconstitute MTG by adding 170 µL deionized water.

The reconstituted reagents are stable for several hours at 2 – 8 °C. For extended storage freeze reconstituted components at -20 °C.

d) Prepare at least 900 µL stock solution of your protein (up to 1.1 mg/mL).

6.4. Procedure

The steps for protein biotinylation are identical for Q- and K-labeling, if not indicated otherwise. Specific procedure for Q-Biotinylation Kit (L101) is indicated with **Q**, for K-Biotinylation Kit (L201) it is indicated with **K**.
Please protect samples from direct sun light exposure.

6.4.1. Biotinylation Procedure

a) Transfer 900 µL of your target protein (1.1 mg/mL) into a 1.5 mL reaction tube.

b) **Q** Add 50 µL Q-Labeling substrate (SQ-Bio).

K Add 50 µL K-Labeling substrate (SK-Bio).

c) Add 20 µL of MTG solution to start the labeling reaction.

d) Incubate at room temperature in a shaker (~400 rpm) for 30 min.
Note: For some proteins an extended incubation time up to 24 h may be appropriate.

e) Stop the labeling reaction by adding 40 µl MTG-Blocker (Stop) and incubate for 30 min at room temperature.

6.4.2. Removal of Excess Labeling Material

The desalting columns (GPC) provided have a size exclusion limit of 5 kDa, therefore proteins and peptides smaller than 5 kDa cannot be purified with these columns. In this case you need to establish your own clean-up method (see section 6.6).

a) Remove the top and bottom cap. Pour off the storage solution and place the column in a suitable rack (not provided).

b) Equilibrate the purification column (GPC) three times with 5 mL MTG-Labeling Buffer (LB), discard flow through.
Note: Alternatively, you may equilibrate the purification column with a buffer suitable for your protein.

c) Transfer your 1 mL-labeling reaction onto the column. Let the sample enter the column bed by gravity flow, discarde flow through.

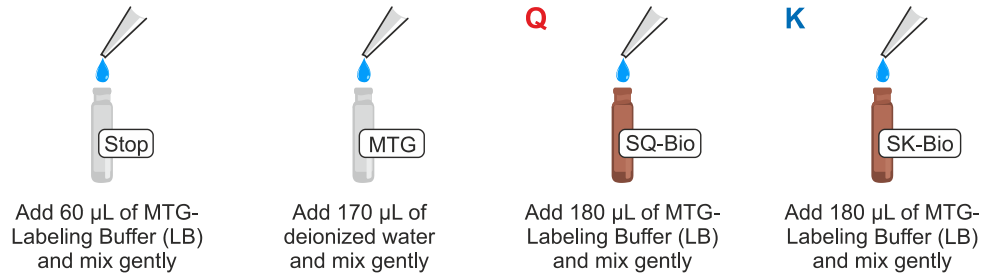
d) Place a collection tube under the column. Elute your protein by addition of 1.5 mL MTG-labeling buffer and collect flow through.
Note: If you equilibrated the column with a different buffer, please use the same buffer for this step.

e) Discard column.

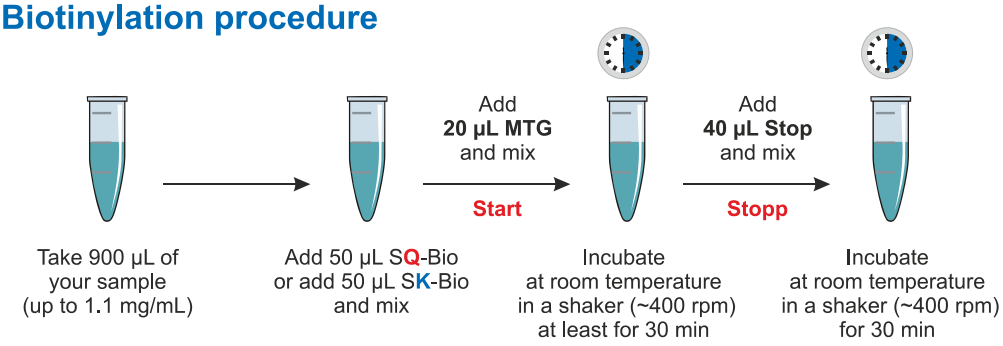
The protein recovery-rate should be >90%, which can be measured by determining protein concentration of your biotinylated protein (e.g. Bradford Protein Assay). Further, analyse your biotinylated protein with appropriate methods established in your lab, e.g. using SDS-PAGE or Western blotting.
Your buffer exchanged biotinylated protein does still contain inhibited MTG. In case your protein contains an affinity tag, you can use affinity chromatography techniques to remove excess labeling material and inhibited MTG instead of this GPC step.

6.5. Procedure Flow Chart

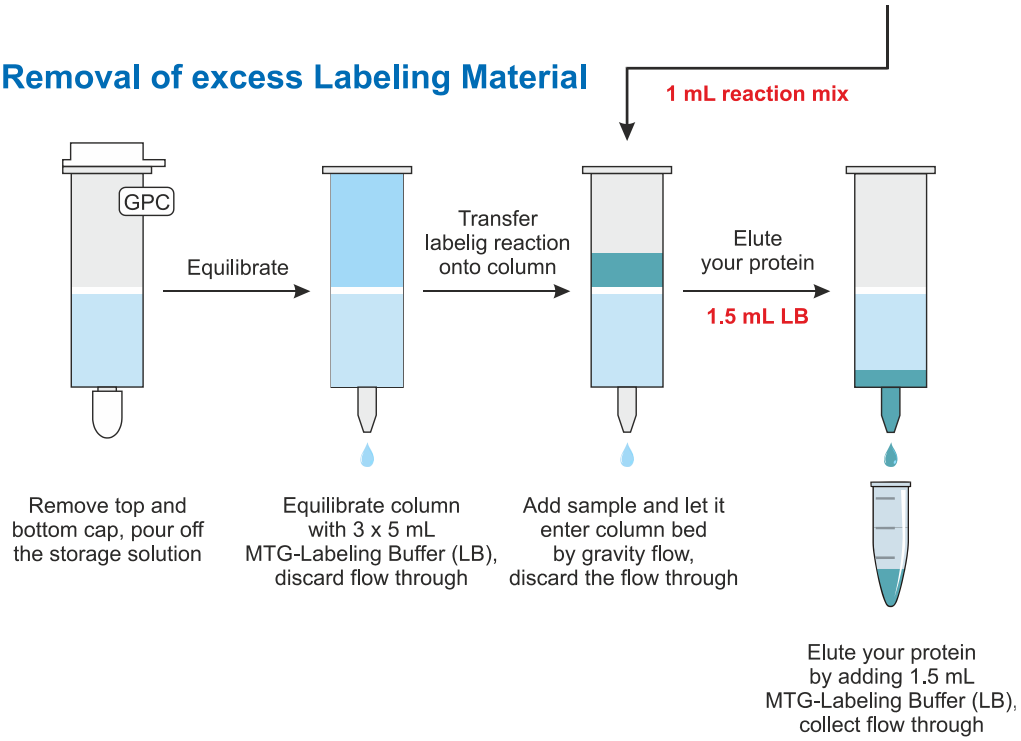
Reagent preparation



Biotinylation procedure



Removal of excess Labeling Material



6.6. Examples

In the following sections some examples for **Q**- and **K**-Biotinylation results are presented. Sample preparation and biotinylation were performed according to the description in sections 6.3 and 6.4.

6.6.1. Biotinylation of Transglutaminase 2

The Substrate Finder Kit (L001) revealed that transglutaminase 2 (TG2) is suitable as a Q- and K-substrate (data not shown). In the following, TG2 was labeled with biotin using the Biotin TGase Protein **K**-Labeling Kit (L201) kit. Three fractions were collected during the desalting process for removal of excess labeling material.

The samples were analysed with SDS-PAGE, Coomassie-staining (Figure 9A) and Western-Blot. As control samples biotinylated TG2, TG2 and MTG (respective lanes 1-3 in Figure 9) were analysed alongside the biotinylation reaction mix and desalting fractions (lanes 4-7 in Figure 9).

Using streptavidin conjugated alkaline phosphatase (Sigma, Figure 9B), biotinylated proteins were detected in lane 1, 4, 5 and 6 corresponding with biotinylated positive control, reaction mixture with all labeling components and desalted target fraction containing about 90% of biotinylated TG2. The following fraction (lane 6) contained some residual protein.

Using a polyclonal antibody to human tissue transglutaminase (A014, Figure 9C), TG2 was detected in lane 1, 2, 4, 5 and 6 at ~ 90 kDa, corresponding to TG2 in the control samples. MTG was detected using a polyclonal antibody to microbial transglutaminase (A019, Figure 9D). The blot shows MTG in lanes 3, 4 and 5 at 38 kDa.

MTG is not separated from TG2 by the desalting process since the columns molecular weight exclusion limit is below 5 kDa.

Looking at lane 4 and 5 in Figure 9B and C, at molecular weights above 170 kDa, bands were detected representing cross-linked TG2. However, the majority of protein was biotinylated, since due to substrate surplus, the biotinylation reaction was favoured.

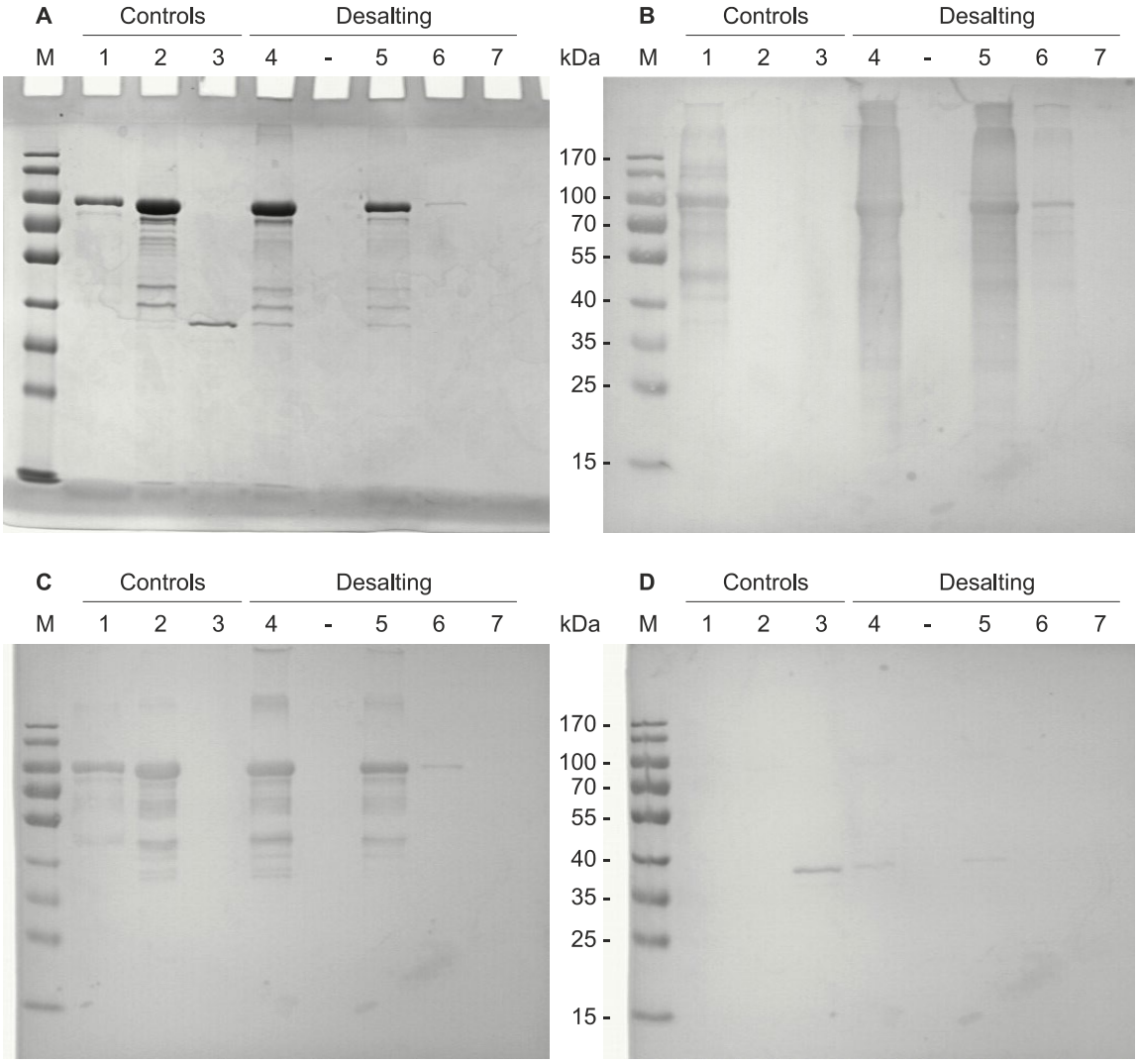


Figure 9: Biotinylation of TG2. A) Coomassie stained SDS-PAGE-gel. B, C, D) Western Blots. B) Detection of Biotin. C) Detection of TG2. D) Detection of MTG. M = Protein Marker; 1-3 Controls: biotinylated TG2, TG2 and MTG; 4 = total reaction mix; 5-7 = Desalting fractions with about 90% TG2 recovery in 5.

7. PEG TGase Protein Q- and K-Labeling Kits for PEGylation

PEG1,088 TGase Protein **Q**-Labeling Kit (L102) and PEG1,088 TGase Protein **K**-Labeling Kit (L202) are intended for MTG mediated labeling of proteins (> 40 kDa) with PEG1,088. The reagents in both kits are sufficient for PEGylation of 5 x 1 mg protein.

PEG5,000 TGase Protein **Q**-Labeling Kit (L103) and PEG5,000 TGase Protein **K**-Labeling Kit (L203) are intended for MTG mediated labeling of proteins (> 5 kDa) with PEG5,000. The reagents in both kits are sufficient for PEGylation of 5 x 1 mg protein.

PEG TGase Protein **Q**-Labeling Kits are suitable for labeling of proteins containg accessible glutamine (Q) residues. Please follow the instructions indicated with **Q**.
PEG TGase Protein **K**-Labeling Kits are suitable for labeling of proteins containg accessible lysine (K) residues. Please follow the instructions indicated with **K**.

Please use the Substrate Finder Kit (L001) initially to determine the suitable PEGylation kit.

The PEGylation protocol is based on 600 µL of your target protein (up to 1.7 mg/mL).
In the first step, your target protein and PEG1,088 or PEG5,000 substrate are mixed. The labeling reaction is started by addition of microbial transglutaminase (MTG). After 60 min, the reaction is stopped by addition of MTG-inhibitor (Stop). **Labeling time may be increased up to 24 h for low reactive proteins.**

In the case of **PEG1,088-substrate**, you can separate your PEGylated protein from excess labeling material by performing a buffer exchange using the provided desalting columns (GPC). The columns have a size exclusion limit of 40 kDa. This step provides the opportunity for a quick and easy buffer exchange into a buffer best suitable for your downstream application.
In the case of **PEG5,000-substrate**, you need to separate your PEGylated protein from excess labeling material **using your own chromatography techniques**. Therefore no GPC comumns are provided.

Labeling performace should be analyzed by SDS-PAGE.

7.1. Content of the Kits

a)	MTG	Microbial Transglutaminase	lyophilized solid	1 vial
b)	LB	MTG-Labeling Buffer (20 mM Tris-HCl, pH7.2, 300 mM NaCl, 0.02% sodium azide)	100 mL	1 bottle
c)	Stop	MTG-Blocker	lyophilized solid	5 vials
d)	S**Q**-1088	PEG1,088 Substrate for Q-Labeling	white powder	5 vials in kit L102
	S**Q**-5000	PEG5,000 Substrate for Q-Labeling	white powder	5 vials in kit L103
	These components are in the following abbreviated as S**Q**-PEG.			
	S**K**-1088	PEG1,088 Substrate for K-Labeling	20 µL	5 vials in kit L202
	S**K**-5000	PEG5,000 Substrate for K-Labeling	20 µL	5 vials in kit L203
	These components are in the following abbreviated as S**K**-PEG.			
e)	GPC	Purification column	--	5 columns in kit L102 and L202

7.2. Materials Required but not Supplied

a) 1.5 mL Low Bind reaction tubes (e.g. Eppendorf)

b) Pipettes for 10, 100 and 1000 µL and tips

c) Shaker for 1.5 mL reaction tubes

d) Centrifuge with swing-bucket rotor for 15 mL reaction tubes

e) Collection tubes for purification columns (15 mL reaction tubes are suitable)

7.3. Reagent and Sample Preparation

Before opening and using the components, they must have reached ambient temperature (23 ± 3 °C). Do not pool components from different kits, due to possible different handling of the kits. While preparing reagents do not allow foam formation.

Optional: Instead of MTG-Labeling Buffer you may use your own buffer system for reconstituting the reagents and running the labeling reaction. The PEGylation Kits are tested for pH-range 6.0 – 7.5. Beyond this range, please assay the control proteins of Substrate Finder Kit (L001) in order to ensure the kit's performance.

a) **Q** Reconstitute SQ-PEG substrate for Q-Labeling by adding 180 µL MTG-Labeling Buffer (LB) and mix gently.

K Add 180 µL MTG-Labeling Buffer (LB) to SK-PEG substrate for K-Labeling and mix gently.

b) Stop Add 60 µL MTG-Labeling Buffer (LB) to MTG-Blocker and mix gently.

c) MTG Reconstitute MTG by adding 170 µL deionized water.

The reconstituted reagents are stable for several hours at 2 – 8 °C. For extended storage freeze reconstituted components at -20 °C.

d) Prepare at least 600 µL stock solution of your protein (up to 1.7 mg/mL).

7.4. Procedure

The steps for protein PEGylation are identical for Q- and K-labeling, if not indicated otherwise. Specific procedures for Q-PEGylation Kit (L102, L103) are indicated with **Q**, for K-PEGylation Kit (L202, L203) they are indicated with **K**.

7.4.1. PEGylation Procedure

a) Transfer 600 µL of your target protein (1.7 mg/mL) into a 1.5 mL reaction tube.

b) **Q** Add 50 µL Q-Labeling substrate (SQ-PEG).

K Add 50 µL K-Labeling substrate (SK-PEG).

c) Add 20 µL of MTG solution to start the labeling reaction.

d) Incubate at room temperature in a shaker (~400 rpm) for 60 min.
Note: For some proteins an extended incubation time up to 24 h may be appropriate.

e) Stop the labeling reaction by adding 40 µl MTG-Blocker (Stop) and incubate for 30 min at room temperature.

7.4.2. Removal of Excess Labeling Material (L102 and L202)

The desalting columns (GPC) provided have a size exclusion limit of 40 kDa, therefore proteins smaller than 40 kDa cannot be purified with these columns. In this case you need to establish your own clean-up method.
Further, the **columns are not suitable for removing excess PEG5,000 substrate!** Therefore, the following procedure is not applicable for the PEG5,000 TGase Protein Labeling Kits (L103, L203).

a) Remove the bottom cap and losen the top cap.

b) Place the column into a collection tube and centrifuge 2 minutes at 1,000 x *g* to remove the storage solution. Discard flow-through.

c) Add 2.5 mL MTG-Labeling Buffer (LB) on top of the resin and equilibrate the purification column (GPC) by centrifuging 2 minutes at 1,000 x *g*, discard flow through. Repeat this step two times.
The resin should appear white and free of liquid after each centrifugation step.
Note: Alternatively, you may equilibrate the purification column with a buffer suitable for your protein.

d) Transfer GPC into a new collection tube.

e) Transfer your 1 mL-labeling reaction onto the column. Centrifuge 4 minutes at 1,000 x *g* and retain flow-through that contains your sample.

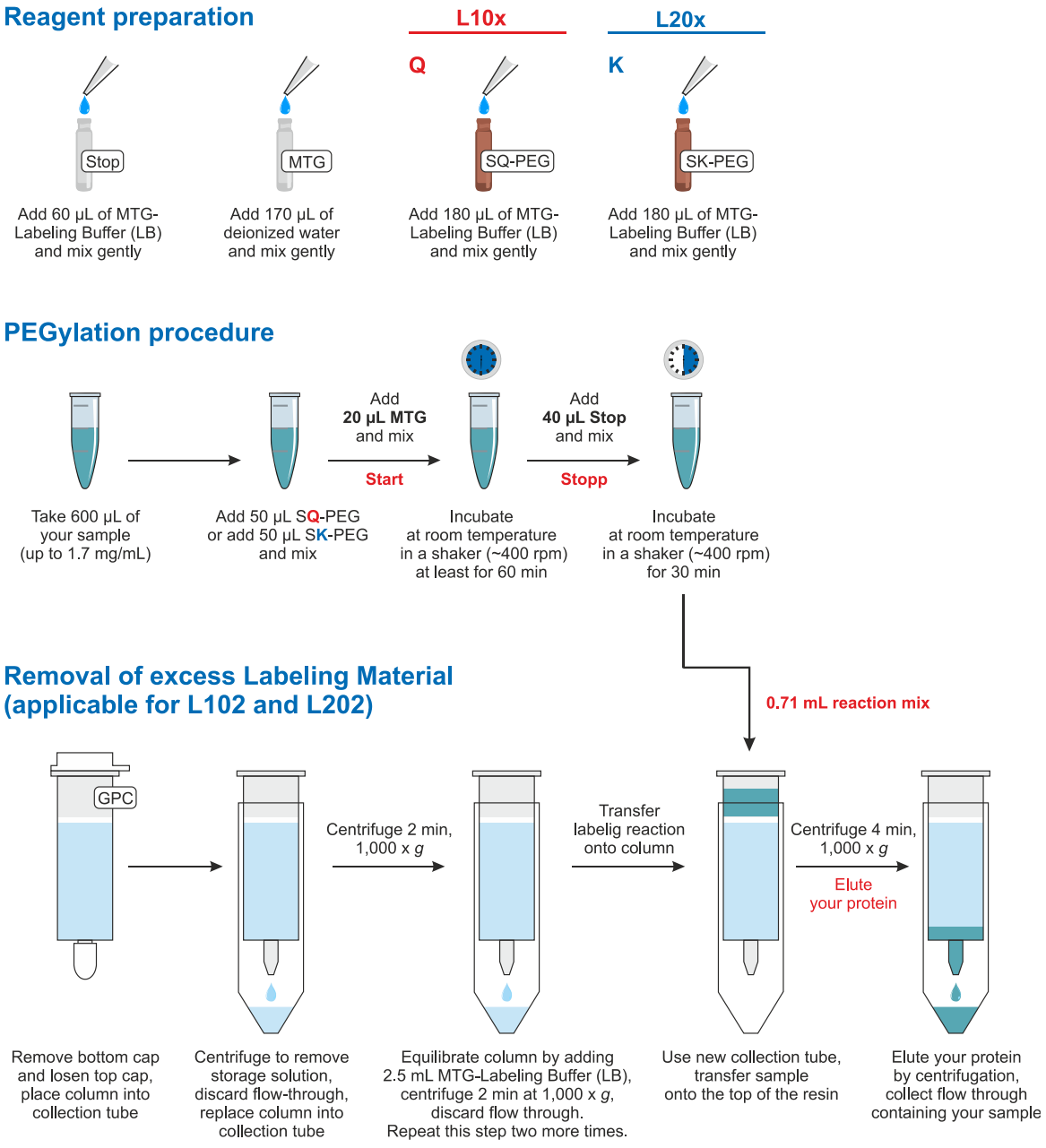
f) Discard column.

The protein recovery-rate should be >90%, which can be measured by determining protein concentration of your PEGylated protein (e.g. Bradford Protein Assay). Further, analyse your PEGylated protein with appropriate methods established in your lab, e.g. using SDS-PAGE.

Your buffer exchanged PEGylated protein does still contain inhibited MTG.

In case your protein contains an affinity tag, you can use affinity chromatography techniques to remove excess labeling material and inhibited MTG instead of this GPC step.

7.5. Procedure Flow Chart



7.6. Examples

Protein PEGylation mediated by microbial transglutaminase (MTG) was tested using the positive control proteins of Substrate Finder Kit (L001).

7.6.1. Q-Labeling

Control protein CQ (Q-substrate Control Protein) contains an amino acid tag rendering the protein to a glutamine-donor protein. Labeling of CQ with amine-donors (PEG1,088)cadaverine or (PEG5,000)cadaverine is shown in Figure 10 (see next page).

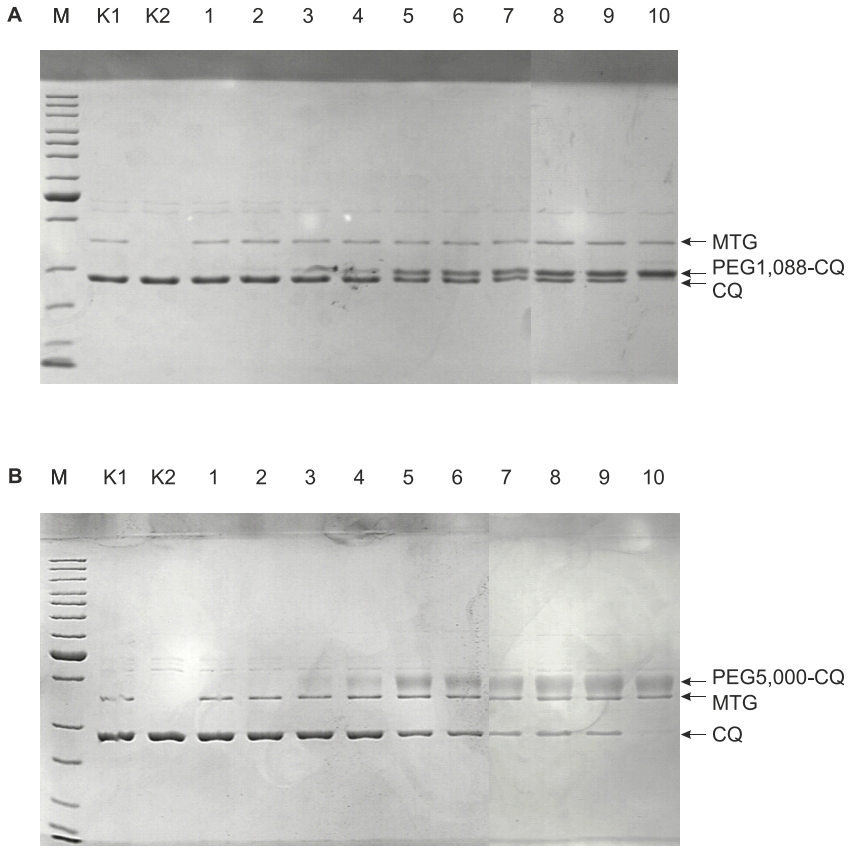


Figure 10: MTG mediated incorporation of PEG derivates into CQ.
A) CQ labeled with (PEG1,088)cadaverine (L102). B) CQ labeled with (PEG5,000)cadaverine (L103). Reaction mixture (RM) contained CQ, PEG derivate and MTG and was incubated at 37°C on a shaker. At several time points samples were taken, mixed with 5x loading dye and heated immediately. Samples were analyzed by SDS-PAGE followed by Coomassie staining. M: Molecular weight marker; K1: RM without PEG derivates; K2: RM without MTG; lanes 1-10: Samples taken after 5 min, 15 min, 30 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min and 16 h reaction time. Arrows: MTG is indicated as well as CQ and PEGylated CQ.

The molecular weight shift of CQ (28 kDa) indicates PEG-derivate incorporation with increasing reaction time. Over time, the band of CQ decreases, whereas a band at 30 kDa or 40 kDa appears, depending on the respective PEG derivate. After 16 h of incubation, no QC band is visible indicating complete PEGylation with the respective derivate.

It can be noted that PEGylation of CQ requires longer incubation times than the dansylation utilized in the Substrate Finder Kit (L001).

Note: It is not possible to label CK with amine-donors, since CK does not contain any accessible glutamine residues.

7.6.2. K-Labeling

Control protein CK (K-substrate Control Protein) contains an amino acid tag rendering the protein to an amine-donor protein. Labeling of CK with glutamine-donors Z-Gln-Gly-(PEG1,088) or Z-Gln-Gly-(PEG5,000) is shown in Figure 11.The tag used in CK contains several lysin residues. Thus, a higher number of PEG molecules labeled to this protein can be expected.

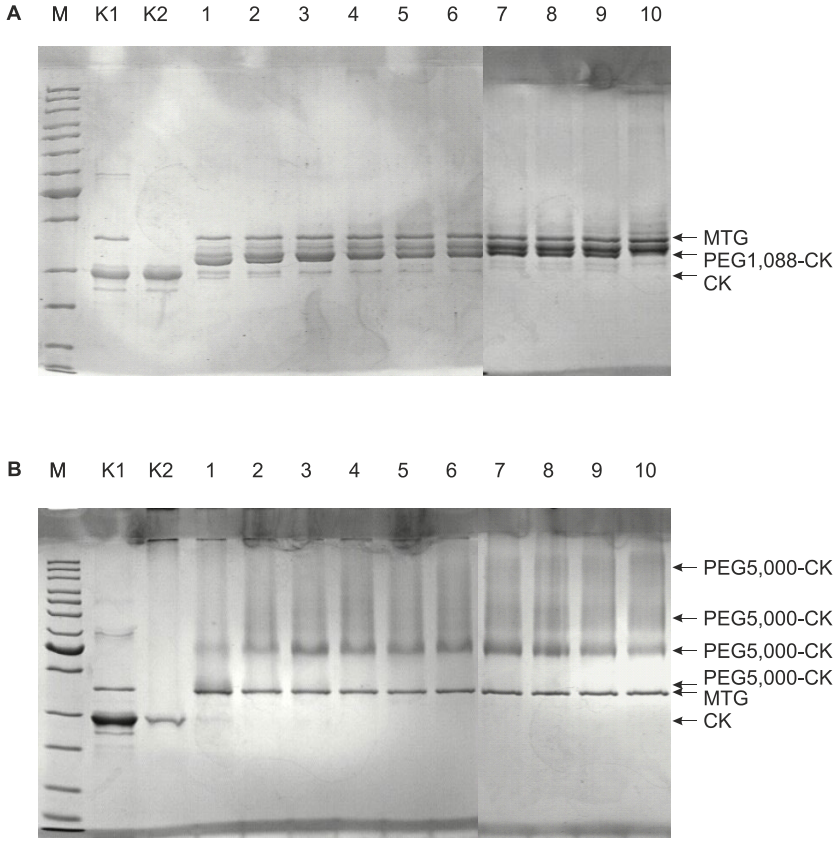


Figure 11: MTG mediated incorporation of PEG derivates into CK.
A) CK labeled with Z-Gln-Gly-(PEG1,088) (L202). B) CK labeled with Z-Gln-Gly-(PEG5,000) (L203). Reaction mixture (RM) contained CK, PEG derivate and MTG and was incubated at 37°C on a shaker. At several time points samples were taken (20 µL), mixed with 5x loading dye (5 µL) and heated immediately. Samples were analyzed by SDS-PAGE followed by Coomassie staining. M: Molecular weight marker; K1: RM without PEG derivates; K2: RM without MTG; lanes 1-10: Samples taken after 5 min, 15 min, 30 min, 60 min, 120 min, 180 min, 240 min, 300 min, 360 min and 16 h reaction time. Arrows: MTG is indicated as well as CK and PEGylated CK.

The molecular weight shift of CK (29 kDa) indicates PEG derivate incorporation with increasing reaction time. Over time, the band of CK decreases, whereas several bands at ~31 kDa or ~60 kDa and higher molecular weights appear, depending on the respective PEG derivate.

After 16 h of incubation, no CK band is visible indicating complete PEGylation with the PEG1,088 derivate. In contrast, already after 15 min of incubation, no CK band is visible indicating complete PEGylation with the PEG5,000 derivate. This increased cross-linking reaction can be attributed to the structure and accessibility of the tag used for CK.

Note: It is not possible to label CQ with glutamine-donors, since CQ does not contain any lysin residues.

8. ATTO-Dye™ TGase Protein Q- and K-Labeling Kits

Zedira offers a variety of fluorescent ATTO-Dye™ TGase Protein **Q**- and **K**-Labeling Kits, which are intended for MTG mediated labeling of proteins (> 5 kDa) with the respective ATTO-Dye™. Reagents in each kit are sufficient for labeling of 1 mg protein. You can label either 1 x 1 mg (▲), 2 x 500 µg (■) or 4 x 250 µg (●) protein per labeling reaction. Please follow the respective symbols in the instruction part.

The selection of ATTO-Dyes™ available is listed in the table below (Table 1 and Table 2).

Table 1: Availabel selection of ATTO-Dyes™ for glutamine (Q) labeling.

The presence of accessible glutamine (Q) amino acid residues may be determined with Substrate Finder Kit (L001). Please choose your kit from the selection below.

ATTO-Dye™	Art. No.	Name
ATTO-488™	L104	ATTO-488™ TGase Protein **Q**-Labeling Kit
ATTO-532™	L105	ATTO-532™ TGase Protein **Q**-Labeling Kit
ATTO-550™	L106	ATTO-550™ TGase Protein **Q**-Labeling Kit
ATTO-647N™	L107	ATTO-647N™ TGase Protein **Q**-Labeling Kit
ATTO-700™	L108	ATTO-700™ TGase Protein **Q**-Labeling Kit

Table 2: Availabel selection of ATTO-Dyes™ for lysine (K) labeling.

The presence of accessible lysine (K) amino acid residues may be determined with Substrate Finder Kit (L001). Please choose your kit from the selection below.

ATTO-Dye™	Art. No.	Name
ATTO-488™	L204	ATTO-488™ TGase Protein **K**-Labeling Kit
ATTO-532™	L205	ATTO-532™ TGase Protein **K**-Labeling Kit
ATTO-550™	L206	ATTO-550™ TGase Protein **K**-Labeling Kit
ATTO-647N™	L207	ATTO-647N™ TGase Protein **K**-Labeling Kit
ATTO-700™	L208	ATTO-700™ TGase Protein **K**-Labeling Kit

ATTO-Dye™ TGase Protein **Q**-Labeling Kits are suitable for labeling of proteins containg accessible glutamine (Q) residues. Please follow the instructions indicated with **Q**.
ATTO-Dye™ TGase Protein **K**-Labeling Kits are suitable for labeling of proteins containg accessible lysine (K) residues. Please follow the instructions indicated with **K**.

Please use the Substrate Finder Kit (L001) initially to determine the suitable labeling kit.

The labeling protocol is based on 500 µL labeling reaction containing up to 1 mg of your target protein.
In the first step, your target protein and ATTO-Dye™ substrate are mixed. The labeling reaction is started by addition of microbial transglutaminase (MTG). After 30 min, the reaction is stopped by addition of MTG-inhibitor (Stop). **Labeling time may be increased up to 24 h for low reactive proteins.**
To separate your ATTO-Dye™ labeled protein from excess labeling material, a buffer exchange is performed using a desalting column (GPC). The column has a size exclusion limit of 5 kDa. This step provides the opportunity for a quick and easy buffer exchange into a buffer best suitable for your downstream application.
Labeling performace should be analyzed by SDS-PAGE and fluorescent analysis.

8.1. Content of the Kits

a)	MTG	Microbial Transglutaminase	lyophilized solid	1 vial
b)	LB	MTG-Labeling Buffer (20 mM Tris-HCl, pH7.2, 300 mM NaCl, 0.02% sodium azide)	50 mL	1 bottle
c)	Stop	MTG-Blocker	lyophilized solid	1 vial
d)	S**Q**-488	ATTO-488™ Substrate for Q-Labeling	white powder	1 vial in kit L104
	S**Q**-532	ATTO-532™ Substrate for Q-Labeling	white powder	1 vial in kit L105
	S**Q**-550	ATTO-550™ Substrate for Q-Labeling	white powder	1 vial in kit L106
	S**Q**-647N	ATTO-647N™ Substrate for Q-Labeling	white powder	1 vial in kit L107
	S**Q**-700	ATTO-700™ Substrate for Q-Labeling	white powder	1 vial in kit L108

These components are in the following abbreviated as S**Q**-ATTO.

S**K**-488	ATTO-488™ Substrate for K-Labeling	20 µL	1 vial in kit L204
S**K**-532	ATTO-532™ Substrate for K-Labeling	20 µL	1 vial in kit L205
S**K**-550	ATTO-550™ Substrate for K-Labeling	20 µL	1 vial in kit L206
S**K**-647N	ATTO-647N™ Substrate for K-Labeling	20 µL	1 vial in kit L207
S**K**-700	ATTO-700™ Substrate for K-Labeling	20 µL	1 vial in kit L208

These components are in the following abbreviated as S**K**-ATTO.

e) GPC Purification column -- 4 columns

8.2. Materials Required but not Supplied

a) 1.5 mL Low Bind reaction tubes (e.g. Eppendorf)

b) Pipettes for 10, 100 and 1000 µL and tips

c) Shaker for 1.5 mL reaction tubes

d) Rack for purification columns

e) Waste beaker

8.3. Reagent and Sample Preparation

Before opening and using the components, they must have reached ambient temperature (23 ± 3 °C). Do not pool components from different kits, due to possible different handling of the kits. While preparing reagents do not allow foam formation.

Optional: Instead of MTG-Labeling Buffer you may use your own buffer system for reconstituting the reagents and running the labeling reaction. The ATTO-Dye™ TGase Protein Labeling Kits are tested for pH-range 6.0 – 7.5. Beyond this range, please assay the control proteins of Substrate Finder Kit (L001) in order to ensure the kit's performance.

a) **Q** Reconstitute SQ-ATTO substrate for Q-Labeling by adding 60 µL MTG-Labeling Buffer (LB) and mix gently.

K Add 60 µL MTG-Labeling Buffer (LB) to SK-ATTO substrate for K-Labeling and mix gently.

b) Stop Add 60 µL MTG-Labeling Buffer (LB) to MTG-Blocker and mix gently.

c) MTG Reconstitute MTG by adding 170 µL deionized water.

The reconstituted reagents are stable for several hours at 2 – 8 °C. For extended storage freeze reconstituted components at -20 °C.

d) Prepare a stock solution of your protein:

- ▲ 400 µL with up to 2.6 mg/mL for labeling of 1x 1 mg protein
- ■ 450 µL with up to 1.2 mg/mL for labeling of 2x 500 µg protein
- ● 480 µL with up to 0.6 mg/mL for labeling of 4x 250 µg protein

8.4. Procedure

The steps for protein labeling with ATTO-Dyes are identical for Q- and K-labeling, if not indicated otherwise. Specific procedure for Q-Labeling Kits (L104, L105, L106, L107, L108) are indicated with **Q**, for K-Labeling Kits (L204, L205, L206, L207, L208) they are indicated with **K**.

Please protect samples from direct sun light exposure.

8.4.1. Labeling Procedure

a) Transfer your target protein into a 1.5 mL reaction tube.

- ▲ 390 µL of your 2.6 mg/mL stock solution for labeling of 1 mg protein
- ■ 445 µL of your 1.2 mg/mL stock solution for labeling of 500 µg protein
- ● 475 µL of your 0.6 mg/mL stock solution for labeling of 250 µg protein

b) **Q** Add Q-Labeling substrate (SQ-ATTO).
K Add K-Labeling substrate (SK-ATTO).

- ▲ 50 µL for labeling of 1 mg protein
- ■ 25 µL for labeling of 500 µg protein
- ● 10 µL for labeling of 250 µg protein

c) Add MTG solution to start the labeling reaction.

- ▲ 20 µL for labeling of 1 mg protein
- ■ 10 µL for labeling of 500 µg protein
- ● 5 µL for labeling of 250 µg protein

d) Incubate at room temperature in a shaker (~400 rpm) for 30 min.
Note: For some proteins an extended incubation time up to 24 h may be appropriate.

e) Stop the labeling reaction by adding MTG-Blocker (Stop) and incubate for 30 min at room temperature.

- ▲ 40 µL for labeling of 1 mg protein
- ■ 20 µL for labeling of 500 µg protein
- ● 10 µL for labeling of 250 µg protein

8.4.2. Removal of Excess Labeling Material

The desalting columns (GPC) provided have a size exclusion limit of 5 kDa, therefore proteins and peptides smaller than 5 kDa cannot be purified with these columns. In this case you need to establish your own clean-up method.

a) Remove the top and bottom cap. Pour off the storage solution and place the column in a suitable rack (not provided).

b) Equilibrate the purification column (GPC) four times with 2 mL MTG-Labeling Buffer (LB), discard flow through.
Note: Alternatively, you may equilibrate the purification column with a buffer suitable for your protein.

c) Transfer your 0.5 mL-labeling reaction onto the column. Let the sample enter the column bed by gravity flow, discard flow through.

d) Place a collection tube under the column. Elute your protein by addition of 1 mL MTG-labeling buffer and collect flow through.
Note: If you equilibrated the column with a different buffer, please use the same buffer for this step.

e) Discard column.

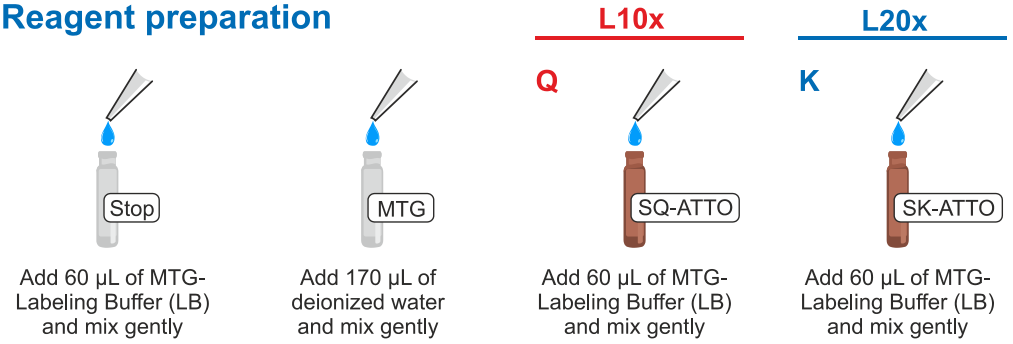
The protein recovery-rate should be >85%, which can be measured by determining protein concentration of your labeled protein (e.g. Bradford Protein Assay). Further, analyse your labeled protein with appropriate methods established in your lab, e.g. using SDS-PAGE or Western blotting.

Your buffer exchanged labeled protein does still contain inhibited MTG. In case your protein contains an affinity tag, you can use affinity chromatography techniques to remove excess labeling material and inhibited MTG instead of this GPC step.

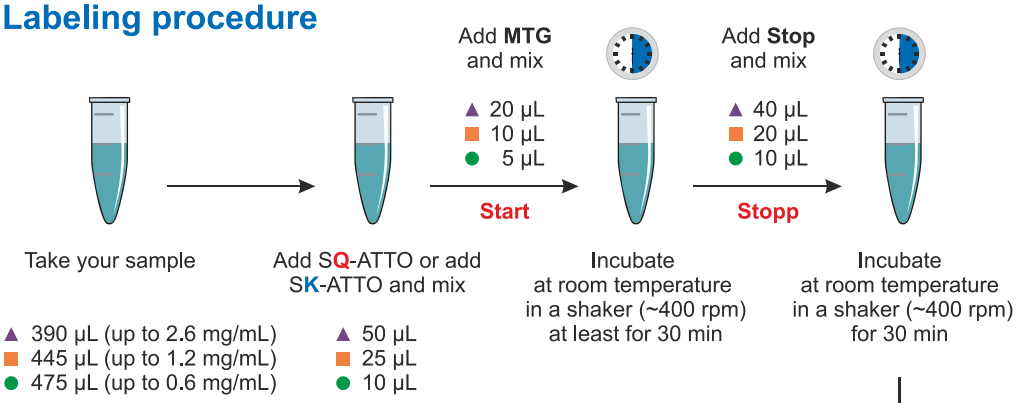
8.5. Procedure Flow Chart

Please follow the procedures ▲ for labeling of 1 mg protein, ■ for labeling of 500 µg protein or ● for labeling of 250 µg protein per labeling reaction.

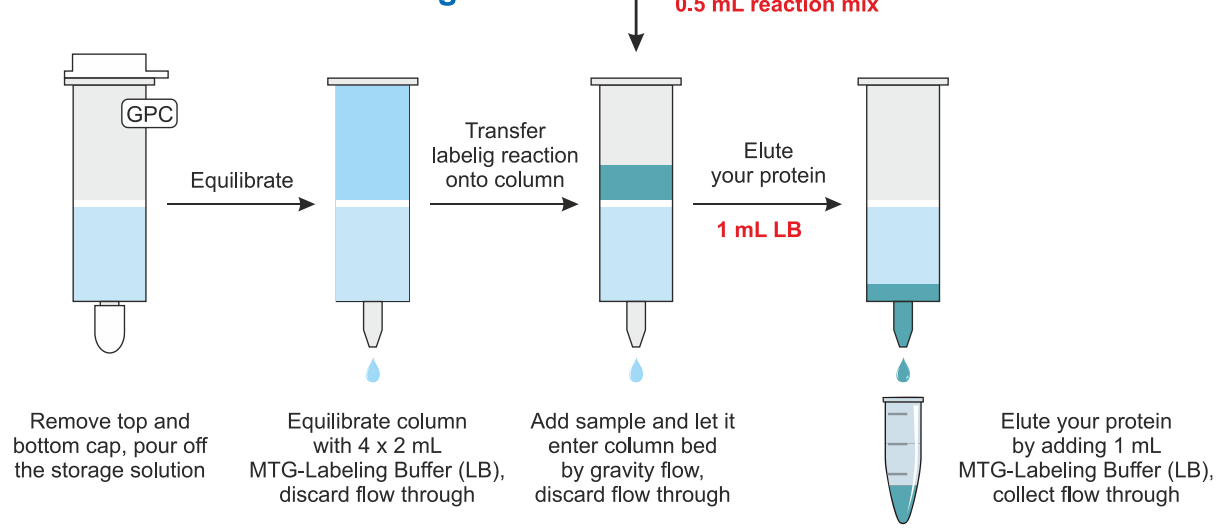
Reagent preparation



Labeling procedure



Removal of excess Labeling Material



9. Trouble-Shooting

In the following sections all questions and concernes regarding the TGase labeling kits are collected and discussed.

Excess cross-linked target protein

Cross-linked protein indicates the target protein entails for MTG accessible Q- and K-residues.

Increase your labeling-substrate concentration (may be ordered separately) and reduce MTG concentration. Further the reduction of labeling time may reduce cross-linking.

No labeled target protein

If your protein could not be labeled with TGase Protein Labeling Kits, you should evaluate your protein's MTG substrate properties using the Substrate Finder Kit (L001). The result should validate your choice of the right labeling kit.

Further increasing labeling reaction time (up to 24 h) and / or reaction temperature (up to 37°C) may improve labeling efficiency.

9.1. Additional Remarks

Labeling of proteins <5 kDa

Due to the size exclusion limit of the provided desalting column, proteins with a molecular weight of < 5 kDa cannot be purified. In this case we recommend using your own established chromatograpy techniques. If your sample contains an affinity tag, please use your affinity chromatography technique. This would also separate residual inhibited MTG.

Removal of residual inhibited MTG

Using the provided desalting column only separates low molecular weight molecules (< 5 kDa), but not residual inhibited MTG.

If you want to remove MTG from your preparation, please use your own chromatography techniques, e.g. affinity chromatography if your protein contains an affinity tag; ion exchange chromatographie or protein A purification for antibodies.

10. Note on Additional Experimental Procedures

In the following you find some hints and recommendations regarding procedures not covered by this manual. Required safety precautions need to be established according to manufacturers guidelines.

10.1. Bradford Protein Assay

In case of unknown protein concentration, we recommend that the protein concentration of the sample is assayed using the method according to Bradford (1976).
Certainly you can use any other method established in your lab in order to determine the protein concentration.

10.2. Concentration of Proteins

The labeling protocols are designed for samples with protein concentrations of 1 mg/mL. If your sample's concentration is below 1 mg/mL you may want to concentrate your protein first, e.g. by ultrafiltration. Be aware of the molecular weight cut off limits, your sample volume, as well as different membrane types available.

10.3. SDS-PAGE

We recommend SDS-PAGE according to the Laemmli protocol for separation of protein samples according to their molecular weight (Laemmli 1970).
The recommended polyacrylamide concentration in the gel is 10% but can be adapted according to the molecular weight of your protein of interest.
For sample preparation, mix 20 µL of your sample with 5 µL of 5x loading dye and heat 10 minutes at 100 °C.
Load 5 µL of each gel sample onto PAGE-gel. Separate the samples electrophoretically at 200 V in the dark for 40-45 minutes.

10.4. UV-Detection of PAGE-gels

Wear eye protection!
Place the unstained gel onto the UV bank to detect the fluorescent dansyl-group. UV signal depends on the settings of the UV-table. Please test several settings, use control proteins as indicator for UV-illumination.

10.5. Coomassie-Staining

The dye Coomassie Brilliant Blue R-250 binds kationic, non-polar, hydrophobic side chains in proteins and can be used for staining techniques (Neuhoff et al. 1988). The detection limit is about 200 ng protein per band.
Please perform the staining according to your established techniques or refer to Brunelle and Green (2014).

11. Warranty

Zedira GmbH makes no warranty of any kind, either expressed or implied, except that the test kit has been thoroughly tested to ensure that its properties specified herein are fulfilled. There is no warranty of the merchantability of this product or of the fitness of the product for any purpose.
The performance data presented here were obtained using the procedure indicated. Any modification in the procedure may affect the results in which case Zedira GmbH disclaims all warranties whether expressed, implied or statutory. Moreover, Zedira GmbH accepts no liability for any damage, whether direct, indirect or consequencial, which results from inappropriate use or storage of the product.

12. Publication bibliography

Bradford, Marion M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. In *Analytical biochemistry* 72 (1), pp. 248–254.

Brunelle, Julie L.; Green, Rachel (2014): Coomassie blue staining. In *Meth. Enzymol.* 541, pp. 161–167. DOI: 10.1016/B978-0-12-420119-4.00013-6.

Dennler, Patrick; Schibli, Roger; Fischer, Eliane (2013): Enzymatic antibody modification by bacterial transglutaminase. In *Methods Mol. Biol.* 1045, pp. 205–215. DOI: 10.1007/978-1-62703-541-5_12.

Laemmli, U. K. (1970): Cleavage of structural proteins during the assembly of the head of bacteriophage T4. In *Nature* 227 (5259), pp. 680–685.

Neuhoff, V.; Arold, N.; Taube, D.; Ehrhardt, W. (1988): Improved staining of proteins in polyacrylamide gels including isoelectric focusing gels with clear background at nanogram sensitivity using Coomassie Brilliant Blue G-250 and R-250. In *Electrophoresis* 9 (6), pp. 255–262. DOI: 10.1002/elps.1150090603.