

Directions for use

ZediXplore EasyGel Kit

Kit for Preparation of Crosslinked Gelatin Hydrogels
using X Pure Gelatin[®] and Andracon[®]

Art.-No. E030

For Research & Development Only

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1. Introduction and background

The ZediXplore EasyGel Kit contains the materials required for preparing stable, crosslinked, gelatin-based gels.

Both components, X Pure Gelatin[®] and Microbial Transglutaminase Andracon[®], have been tested for endotoxins.

The kit enables the generation of X Pure Gelatin[®] gels, which are stabilized by Andracon[®] through the formation of covalent crosslinks.

The conditions given below are recommended as a starting point for preparing crosslinked gelatin gels. Adaptation of these conditions to fulfil the requirements of specific applications may be necessary.

The reaction buffer is not included in the kit. Typically, the buffer which is part of the subsequent application can be used, which is often phosphate buffered saline, pH 7.2.

Interested in the reaction mechanism of Andracon[®]?

Please watch our explanation video on YouTube.



2. Contents of the kit

All components are delivered freeze-dried and must be reconstituted according to the 'Reagent preparation and procedure' section.

- X 4 x 250 mg X Pure Gelatin® (ultra-purified clinical grade gelatin)
- A 1 x 25 U Andracon® (Microbial Transglutaminase, MTG)

3. Materials required but not supplied

- Recommended buffer: PBS buffer pH 7.2
- Standard laboratory equipment

4. Storage and stability

Store Andracon® (A) at $\leq -20^{\circ}\text{C}$ (shipment at ambient temperature is possible).

Store X Pure Gelatin® (X) at room temperature.

Unopened reagents are at least stable until the retest date printed on the box.

5. Reagent preparation and procedure

Do not exchange or pool corresponding components from different kits.

Before starting, all components of the kit must have reached room temperature ($23 \pm 3^\circ\text{C}$).

Note: Depending on your intended use it may be necessary to perform all steps in a sterile environment using sterile buffers and materials.

1. Add the required volume of buffer to one tube of X Pure Gelatin[®] (X).

The examples below are just for guidance. For specific applications, other concentrations may be appropriate.

Gelatin concentration	2,5% (w/v)	5% (w/v)	10% (w/v)
Buffer/water	10 mL	5 mL	2.5 mL

2. Melt the X Pure Gelatin[®] (X) at 70°C for 1 h, e.g. in a thermomixer or in a water bath. Shake gently.
3. Cool the melted X Pure Gelatin[®] (X) down to 40°C , e.g. in a thermomixer or in a water bath. Shake gently.
4. Reconstitute component Andracon[®] (A) by adding 100 μL sterile pure water and shake gently. The stock solution has a volumetric activity of $\sim 250 \text{ U/mL}$ ¹.
 - a. Prepare 4 working aliquots of the non-diluted component A in sterile tubes (tubes not supplied with the kit!).
 - b. Store one Andracon[®] aliquot at room temperature and store the remaining aliquots at -20 to -80°C .
5. Prepare a 1:10 dilution of Andracon[®] (A) by adding 50 μL Andracon[®] (A) to 450 μL buffer (e.g. PBS).

¹ Resulting from overfilling the activity may be higher. For fine tuning of the Andracon[®]-concentration the exact concentration of the enzyme can be found in the CoA. Further, the activity may be determined with ZediXclusive Microbial Transglutaminase Assay Kit (Z009).

6. Prepare the reaction solution by adding Andracon[®] (A, diluted 1:10) to one tube of X Pure Gelatin[®] (X).

It is recommended to start with a concentration of 2.5 U/g Andracon[®] (A) by adding 25 µL Andracon[®] (A, diluted 1:10) to one tube of X Pure Gelatin[®] (X). The Andracon[®] volume is independent from the gelatin concentration due to the same amount of X Pure Gelatin[®] (250 mg) in each tube.

Depending on the specific application, other Andracon[®] concentrations may be appropriate.

7. Immediately, pour the reaction solution into the desired mold (not included in the kit), prewarmed to 37 – 40°C.

Note: gel formation begins when the gelatin cools down below 37°C. Therefore, it is important to pour the solution into the mold rapidly, and to avoid cooling below 37°C.

8. Incubate the mixture for 16 h at 37 – 40°C.
9. Cool down to room temperature (20 – 25°C).
10. Wash the crosslinked gelatin with excess buffer of your subsequent application (not provided with the kit), e.g. 3 times with 10 volumes of buffer for 1 h each.

6. Application Note

In collaboration with **Rheolution Inc.**, **Rousselot Biomedical** and Zedira, the Application Note “Gelatin Crosslinking with Microbial Transglutaminase: Viscoelastic Characterization” was published.

It describes how **Andracon**[®] (Microbial Transglutaminase, MTG) enables precise, efficient, and reproducible crosslinking of **X Pure Gelatin**[®] (ultra-purified clinical grade gelatin), and how the **ElastoSens**[™] **Bio** platform provides real-time insight into gelation kinetics and final mechanical performance.



Access the Application Note on Rheolution's website using this QR-code.



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