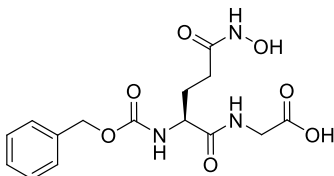


Product number **Z018**  
Revision number **RN3.1**

|                           |  |
|---------------------------|--|
| <b>Product Name</b>       | Z-Glutamyl( $\gamma$ -hydroxamate)-glycine<br>Z-Glu( $\gamma$ -hydroxamate)-Gly-OH   |
| <b>Application</b>        | <p>Reference substance to determine the concentration of product formed by microbial transglutaminase (MTG).</p> <p>The standard hydroxamate assay uses Z-Gln-Gly-OH as peptidic glutamine substrate and hydroxylamine as amine donor. In the presence of MTG, hydroxylamine is enzymatically incorporated into the peptide to form Z-Glutamyl(<math>\gamma</math>-hydroxamate)-glycine. The hydroxamate forms a red colored complex with iron (III) ions quantified at 525 nm.</p> <p>One unit of microbial transglutaminase activity is defined as the amount of enzyme, which causes the formation of 1.0 <math>\mu</math>mole of hydroxamate per minute at 37°C (Folk and Cole, 1966).</p> <p>Z018 represents the reaction product to be measured by the chromogenic endpoint assay, allowing the determination of a calibration curve. For each setting, molar attenuation coefficient (<math>\epsilon</math>) needs to be determined individually.</p> <p>We recommend using <b>Z018</b> to replace <b>G048</b>, which represents the glutamyl(<math>\gamma</math>-hydroxamate) as a surrogate only.</p> |
| <b>Molecular Formula</b>  | $C_{15}H_{19}N_3O_7$   |
| <b>Molecular Weight</b>   | 353.33   |
| <b>Chemical Structure</b> |   |
| <b>Purity by HPLC</b>     | >95 %  |
| <b>Solubility</b>         | <p>&gt;50 mM in buffer "Reagent 1", see page 2</p> <p>Dissolve e.g. 10 mg (28.3 <math>\mu</math>mol) of Z018 in 566 <math>\mu</math>L of aqueous buffer (Reagent 1, see page 2) to obtain a 50 mM (17.7 mg/mL) stock solution.</p> <p><b>NOTE:</b> The solubility of Z018 is not fully investigated. Z018 is not soluble in pure water. Also, solubility seems to be pH dependent. We recommend checking the solubility of e.g. 1 mg of Z018 in the buffer to be used before the experiment.</p>   |
| <b>Appearance</b>         | White solid  |
| <b>Storage</b>            | Store at -20°C, desiccate  |
| <b>Related products</b>   | <p>T001 - Recombinant microbial (bacterial) transglutaminase</p> <p>Z009 - ZediXclusive Microbial Transglutaminase Assay Kit</p> <p>C001 - Z-Gln-Gly-OH</p>  |
| <b>Release date</b>       | 16 January 2025  |
| <b>NOTE</b>               | INTENDED FOR RESEARCH USE ONLY, NOT FOR USE IN HUMAN, THERAPEUTIC OR DIAGNOSTIC APPLICATIONS.  |

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## Exemplary determination of $\epsilon$

The molar attenuation (extinction) coefficient ( $\epsilon$ ) was determined using a serial dilution of Z018 ranging from 1.9 to 0.05 mM in duplicates at ambient temperature. Briefly, a 40 mM stock solution of Z-Glu( $\gamma$ -hydroxamate)-Gly-OH (Z018) was dissolved and diluted in Reagent 1 (0.2 M TRIS, 0.1 M hydroxylammonium chloride, 10 mM glutathione, pH 6.0). Subsequently, another 500  $\mu$ l of Reagent 1 were combined with 50  $\mu$ l of each Z018 dilution described above.

By adding 500  $\mu$ l of the stop solution consisting of equal volumes of 12% HCl, 50 g/l FeCl<sub>3</sub> in 0.1N HCl and 12 % trichloroacetic acid, the hydroxamate forms a red colored complex with iron (III) ions, quantified at 525 nm (Fig. 1).

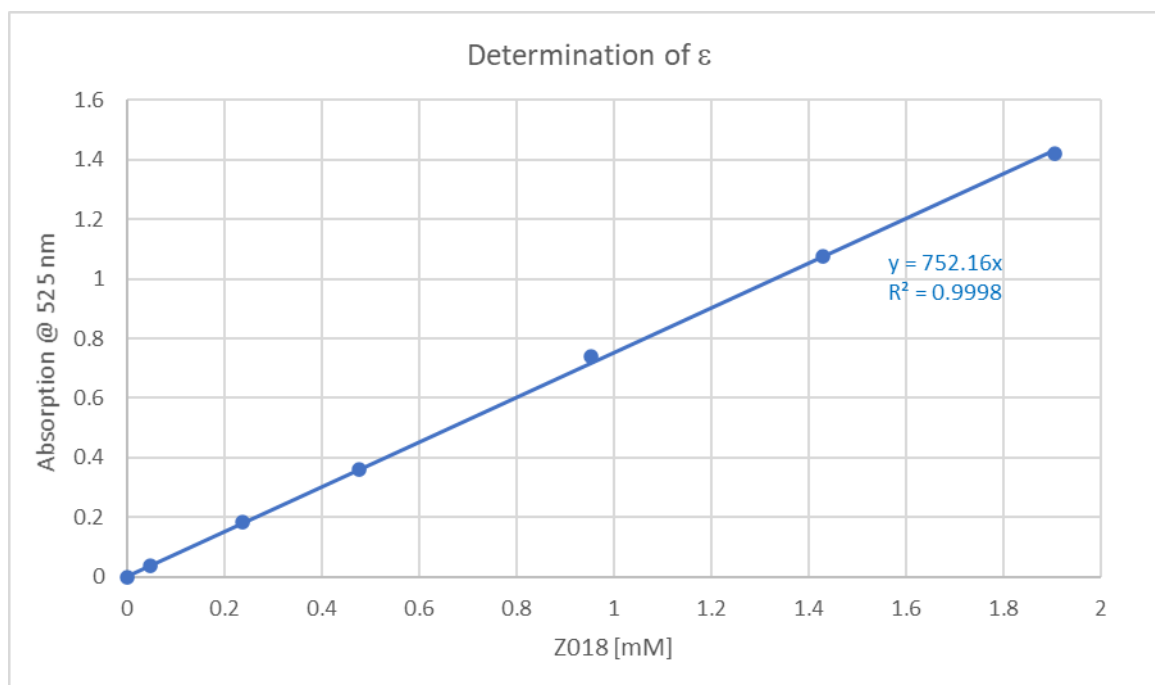


Fig. 1: Determination of molar attenuation (extinction) coefficient  $\epsilon = 0.75 \text{ ml}/(\mu\text{mol}\cdot\text{cm})$