Product Information

Microbial Transglutaminase

Lyophilized powder, ≥12 units/mg protein

SAE0159

Product Description

E.C. (Enzyme Commission) Number: 2.3.2.13

CAS Number: 80146-85-6

Molecular Weight: 38,333 Da

Isoelectric Point (pI): 8.9

Transglutaminases are a family of enzymes that catalyze isopeptide bond formation. This bond formation occurs between the γ-carboxyamide group of glutamine and various primary amines (primarily the ε-amino group of lysine). The resulting intermolecular or intramolecular cross-linking is highly stable and shows high resistance to proteolytic degradation.

Reagent

Specific activity: ≥ 12 units/mg protein

Activity: ≥ 2 units/mg solid

Purity: ≥95% (SDS-PAGE)

Excipients present: lactose, sodium acetate, sodium chloride

Storage/Stability

Store the product at –20 °C. It is recommended to store the reconstituted protein in working aliquots at –20 °C to avoid repeated freeze-thaw cycles.

Precautions and Disclaimer

For R&D use only. Not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Procedure

In the following sample protocol, microbial transglutaminase (mTG) is used to crosslink dansylcadaverine via its primary amine to glutamine residues on a deglycosylated monoclonal antibody. While these conditions can be applied to other proteins, researchers are advised to optimize protocols and conditions for their specific application.

Reagent Preparation

mTG Reconstitution Buffer: 20 mM Tris-HCl, 150 mM NaCl, in Molecular Biology grade water; pH 8

mTG Enzyme: Reconstitute product to 0.14 Units/µL in mTG Reconstitution Buffer. Refer to the lot-specific Certificate of Analysis (CofA) for its Unit specific activity value.

mAb Solution: Make a solution of the antibody (or other protein) of choice at ~10 mg/mL in mTG Reconstitution Buffer.

Note: The following protocol was established using a deglycosylated version of Cat. No. MSQC22. *N*-linked glycosylation at ASN-297 on the mAb Fc region **will** interfere with conjugation.[1,2] This can be removed enzymatically with PNGase F, such as PNGase Fast (Cat. No. EMS0001).

Dansylcadaverine Solution: Prepare 35 mM dansylcadaverine (Cat. No. 30432) solution in DMSO.

Table 1: Reaction Components

Reagent	**Amount/Quantity**
mAb Solution (10 mg/mL)	100 µL
DMSO	10.5 µL
Dansylcadaverine Solution (50 molar equivalents to mAb)	9.5 µL
mTG Reconstitution Buffer	Q.S.* to 200 µL
mTG Enzyme (0.14 U/mL)	17 µL, or as needed†
Total Reaction Volume	**200 µL**

* *Quantum satis* (the amount which is needed)

Protocol

1. Add the reagents listed in Table 1 into the reaction vessel. The mTG enzyme **must be added last**. The final concentration of DMSO is to be ≤ 10%.[1]

Note (†): The amount of substitution can be varied by varying the amount of mTG enzyme added to the reaction. The mTG amount added will depend on the target DAR. Increase the amount for a higher DAR. The 17 µL recommended in Table 1 achieves a DAR of ~1.8 under the conditions provided.

2. Incubate at 37 °C for ~20 hours.
3. The material can now be exchanged into a buffer of choice (such as PBS). The mTG enzyme can then be removed using a concentrator with a nominal MWCO of 50 kDa (such as Cat. No. GE28-9322-36).
4. Measure the Drug Antibody Ratio (DAR) by method of choice such as HIC/HPLC.

Results

Drug to Antibody ratio (DAR) is shown by HPLC using Hydrophobic Interaction Chromatography in Figure 1. The highest possible theoretical DAR for Immunoglobulin-G1 is 2.0. The above protocol can produce a DAR up to 1.8.

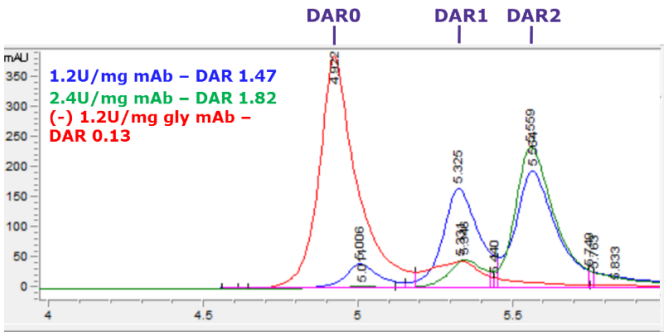

Figure 1. HIC Chromatogram of mAb samples treated with mTG depicting the degree of substitution observed in terms of DAR

- Blue, Green: MSQC22 treated with 1.2 and 2.4 units mTG per mg of MSQC22, respectively.
- Red: Negative control- glycosylated mAb treated with mTG

References

1. Dickgiesser, S. *et al*., *Methods in Mol. Biol*., **2012**, 135-149 (2019).
2. Dennler, P. *et al*., *Bioconjugate Chem*., **25(3)**, 569-578 (2014).

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SAE0159pis Rev 02/22 MP,SBC,SM,GCY