Product Information

Enhanced Microbial Transglutaminase

Glycosylation Tolerant, for Site-Specific Antibody Bioconjugation

SAE0217

Product Description

Synonyms: Protein-Glutamine-γ-Glutamyltransferase, Protein-glutamine:amine γ-glutamyltransferase, eMTG

E.C. (Enzyme Commission) Number: 2.3.2.13

CAS Number: 80146-85-6

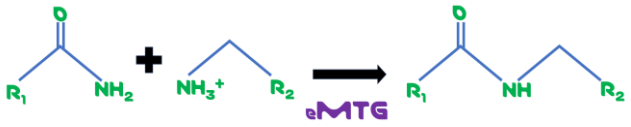
Molecular Weight: 38.0 kDa

Isoelectric Point (pI): 7.79

Enhanced Microbial Transglutaminase (eMTG) is an enzyme that catalyzes the formation of new isopeptide bonds between the primary amine of a drug linker and glutamine residues of a protein. This engineered form is designed specifically for conjugation to IgG-type antibodies at position Q295 to produce site-specifically conjugated antibody-drug conjugates (ADCs). Unlike other transglutaminases eMTG is tolerant of glycosylation at N297 of the IgG-Fc domain.

eMTG provides one-step, site-specific conjugation of native antibodies to produce homogenous ADCs and bioconjugates with DAR of 2 (or more depending on the chosen linker). No glycan engineering or removal is required, preserving the native antibody and glycosylation. With compatible primary amine-containing drug linkers, the bioconjugation reaction proceeds simply in one step.

Reaction



eMTG catalyzes the reaction shown above, and is tolerant of N297 glycosylation on Immunoglobulin G, where R1 = Protein (via glutamine amino acid) and R2 = payload linker.

Reagent

- Specific activity: ≥ 30 units/mg protein
- Purity: ≥ 95% (SEC-HPLC)
- Excipients present: HEPES pH 7.0, Trehalose

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, enhanced microbial transglutaminase (eMTG) is used to crosslink drug linker G3-MMAE to Trastuzumab monoclonal antibody (TmAb). While these conditions can be applied to other antibody drug conjugates, researchers are advised to optimize protocols and conditions for their specific application.

Reagent Preparation

eMTG Reconstitution Buffer: pure water

eMTG Enzyme: Reconstitute product to 220 U/mL in pure water to obtain a stock solution of 20 mg/mL of eMTG. See batch specific data from label or CofA for exact Units/mg.

mAb Solution: Make a solution of the antibody of choice at 8-10 mg/mL in 24 mM HEPES buffer pH 7.0.

Protocol

- Conjugation of DAR ≥ 1.8 can be achieved using native antibody and there is no need for glycosylation removal.
- Drug linker G3-MMAE (custom made) Solution: Prepare a 20 mM solution in DMSO 12.9 mg/mL solution in DMSO.
- The molar ratio between drug-linker and antibody should be 12:1.
- 6 molar equivalent of drug linker per conjugation site (typically two conjugation sites).

Table 1: Example Reaction Components

Reagent	Amount/ Quantity	Concentration in final mix
Hepes Buffer pH 7.0 (24 mM)	165.5 µL	7.9 mM
mAb Solution (8.45 mg/mL)	295.9 µL	5 mg/mL
G3-MMAE (20 mM)	8.6 µL	0.344 mM
eMTG (220 U/mL)	30 µL	13.2 U/mL
Total Reaction Volume	500 µL	

1. Add the reagents listed in Table 1 into the reaction vessel.

   Note: The eMTG enzyme must be added last.

   The amount of substitution can be varied by varying the amount of eMTG enzyme added to the reaction. The eMTG amount added will depend on the target DAR. Increase the amount for a higher DAR.

   The amount of eMTG recommended in Table 1 achieves a DAR of ≥ 1.8 at 3 Units eMTG per mg Ab under the conditions provided.
2. Incubate at 37 °C for 18-24 hours.
3. The material can now be exchanged into a buffer of choice (such as PBS). The eMTG enzyme can then be removed using a concentrator with a nominal MWCO of 50 kDa (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 TmAb/Immunoglobulin-G1 is 2.0.

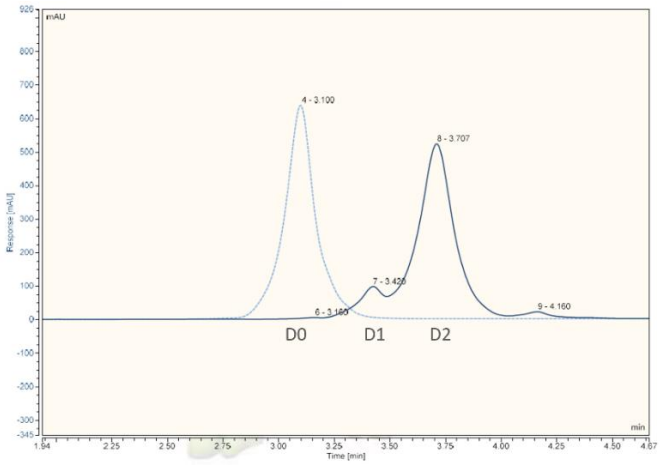


Figure 1. HIC Chromatogram of TmAb sample before and after treatment with eMTG.

- Light blue broken line
  Negative control- intact Trastuzumab monoclonal antibody (TmAb)
- Dark blue line
  MSQC22 treated with 3 units eMTG per mg of MSQC22, DAR 1.96

References

1. S. Dickgiesser, M. Rieker, D. Mueller-Pompalla et. al. (2020) Site-Specific Conjugation of Native Antibodies Using Engineered Microbial Transglutaminases. Bioconjugate Chem. 31, 4, 1070–1076.
2. Dickgiesser, S. et al., (2019) Site-Specific Antibody-Drug Conjugation Using Microbial Transglutaminase. Methods in Mol. Biol., 2012, 135-149.

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