Store at -20°C

PNGase F Kit

#52749

1 Kit

Support: +1-978-867-2388 (U.S.)
cellsignal.com/support

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For Research Use Only. Not for Use in Diagnostic Procedures.

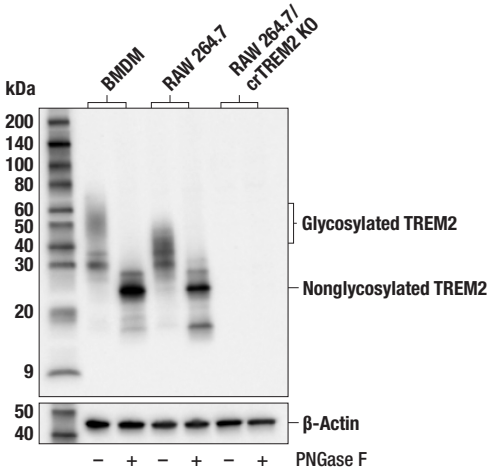
Product Includes	Product #	Kit Quantity	Storage Temp
PNGase F	87622	150 µL	-20°C
GlycoBuffer II (10x)	28106	1 mL	-20°C
Glycoprotein Denaturing Buffer (10x)	43210	1 mL	-20°C
10% NP-40	41129	1 mL	-20°C

Description: The PNGase F Kit provides reagents that, when combined, remove high mannose *N*-glycans from glycoproteins, leaving *N*-glycan core oligosaccharides intact and suitable for further analysis. Peptide N-Glycosidase F (PNGase F) is free of proteases and Endo F activities and is purified from *Flavobacterium meningosepticum*. It cleaves between the innermost GlcNAc and asparagine residues of high mannose, hybrid, and complex oligosaccharides from *N*-linked glycoproteins. The PNGase F Kit can be used under native and denaturing conditions.

Specificity/Sensitivity: The PNGase F Kit effectively removes high mannose *N*-glycans from glycoproteins. It is non-recombinant with no detectable endoglycosidase F1, F2, or F3 contamination.

Source/Purification: The PNGase F component is produced in a strain of *Flavobacterium meningosepticum* and is free of proteases and Endo F activities.

Unit Definition: One unit is defined as the amount of enzyme required to remove > 95% of the carbohydrate from 10 µg of denatured RNase B in 1 hr at 37°C in a total reaction volume of 10 µL.



Western blot analysis of extracts from mouse bone marrow-derived macrophages (BMDM), RAW 264.7, and CRISPR/Cas9 TREM2 knockout (KO) RAW 264.7 cells, untreated (-) or treated with Peptide N-Glycosidase F (PNGase F; +), using TREM2 (E9O9F) Rabbit mAb #59621 (upper) or β-Actin (D6A8) Rabbit mAb #8457 (lower).

Storage: All components in this kit are stable for 24 months when stored at the recommended temperature. *Aliquot to avoid repeated freeze/thaw cycles.*

Please visit cellsignal.com for validation data and a complete listing of recommended companion products.

Directions for Use:

Denaturing Reaction Conditions:

1. Add 1-20 µg of glycoprotein, 1 µL of Glycoprotein Denaturing Buffer (10x), and H_2O (if necessary) to make a total volume of 10 µL.
2. Heat reaction at 100°C for 10 min to denature glycoprotein.
3. Chill reaction on ice and centrifuge for 10 sec.
4. Add 2 µL of GlycoBuffer II (10x), 2 µL of 10% NP-40, and 6 µL of H_2O to make a total reaction volume of 20 µL.
   Note: PNGase F is inhibited by SDS, therefore it is essential to have NP-40 in the reaction mixture under denaturing conditions. Failure to include NP-40 in the denaturing protocol will result in loss of enzymatic activity.
5. Add 1 µL of PNGase F, mix gently.
6. Incubate at 37°C for 1 hr.
7. Analyze by method of choice.
   Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.
   Note: The optimal incubation times and enzyme concentrations must be determined empirically for each individual substrate.

Non-Denaturing Reaction Conditions:

When deglycosylating a native glycoprotein, it is recommended that an aliquot of the glycoprotein is subjected to the denaturing protocol to provide a positive control for the fully deglycosylated protein. The non-denatured reaction can then be compared to the denatured reaction to determine the extent of reaction completion.

1. Add 1-20 µg of glycoprotein, 2 µL of Glycoprotein Denaturing Buffer (10x), and H_2O (if necessary) to make a total volume of 20 µL.
2. Add 2-5 µL of PNGase F, mix gently.
3. Incubate at 37°C for 4-24 hr.
   Note: To deglycosylate a native glycoprotein, longer incubation time as well as more enzyme may be required.
4. Analyze by method of choice.
   Note: The simplest method of assessing the extent of deglycosylation is by mobility shifts on SDS-PAGE gels.

Applications: **W**—Western **IP**—Immunoprecipitation **IHC**—Immunohistochemistry **ChIP**—Chromatin Immunoprecipitation **IF**—Immunofluorescence **F**—Flow cytometry **FC-FP**— Flow cytometry-Fixed/Permeabilized **FC-L**— Flow cytometry-Live **E-P**—ELISA-Peptide
Species Cross-Reactivity: **H**—human **M**—mouse **R**—rat **Hm**—hamster **Mk**—monkey **Mi**—mink **C**—chicken **Dm**—D. melanogaster **X**—Xenopus **Z**—zebrafish **B**—bovine **Dg**—dog **Pg**—pig **Sc**—S. cerevisiae **Ce**—C. elegans **Hr**—Horse
All—all species expected Species enclosed in parentheses are predicted to react based on 100% homology.