SmartEnzymes™

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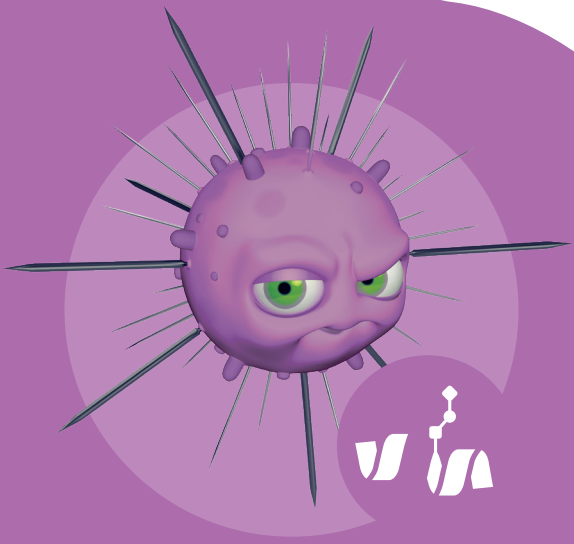

ImpaRATOR™

Lyophilized

STORE AT

-20°C

FOR RESEARCH USE ONLY

Instructions for Use

ImpaRATOR Lyophilized 2000 units (G1-IR1-020)
Process 2 mg O-glycoprotein

DOWNLOAD INSTRUCTIONS FOR USE

www.genovis.com/ifu-G1-IR1

www.genovis.com

Lyophilized Enzyme for O-glycan-specific Protein Digestion

ImpaRATOR is an O-glycan-dependent protease that catalyzes the hydrolysis of the peptide bond adjacent to glycosylated serine or threonine residues in glycoproteins and glycopeptides. It cleaves N-terminally of serine or threonine residues modified with mucin-type O-glycans, including sialylated species.

Mucin-type O-glycans are required for ImpaRATOR activity and the enzyme will not digest unmodified serine or threonine residues, or at N-glycosylation sites of glycoproteins. The enzyme accepts a broad range of O-glycan structures, including sialylated core 1 and core 2 structures as well as the Tn antigen.

ImpaRATOR is derived from *Pseudomonas aeruginosa* and expressed in *E. coli.* The enzyme contains a His-tag and has a molecular weight of 97 kDa.

UNIT DEFINITION

One unit ImpaRATOR Lyophilized digests ≥ 95% of 30 µg etanercept at least at one site when incubated in TBS (50 mM Tris-HCl, 150 mM NaCl), pH 7.6 at 37°C for 30 minutes. For O-glycopeptide mapping applications, one unit ImpaRATOR Lyophilized digests 1 µg O-glycosylated protein when incubated in TBS, pH 7.6 at 37°C for 2 hours.

CONTENT AND STORAGE

ImpaRATOR Lyophilized is supplied lyophilized in TBS (50 mM Tris-HCl, 150 mM NaCl), pH 7.6, with no preservatives added. ImpaRATOR Lyophilized is shipped at ambient temperature, and should be stored at -20°C upon arrival.

After reconstitution, the ImpaRATOR enzyme is stable for at least 1 month at +4-8°C.

ImpaRATOR is for R&D use only.

QUALITY CONTROL

ImpaRATOR Lyophilized is tested to meet the specifications and lot-to-lot consistency.

ImpaRATOR Lyophilized is tested for absence of microbial contamination with blood agar plates, Sabouraud dextrose agar plates and fluid thioglycollate medium.

YOU MIGHT ALSO BE INTERESTED IN

OpeRATOR®
O-glycan specific protein digestion

OmniGLYZOR™
Hydrolysis of N- and mucin-type O-glycans

OglyZOR®
Hydrolysis of core 1 O-glycans

GlycOCATCH®
Enrichment of O-glycopeptides

O-glycan-specific Protein Digestion

PREPARATIONS

Additional Materials Required

- Digestion buffer: TBS (50 mM Tris-HCl, 150 mM NaCl), pH 7.6.[1]

Sample Preparation

Prepare the glycoprotein in the digestion buffer.
The final protein concentration in the digestion reaction should be 0.1-10 mg/ml.

1. Optimization may be required if buffers other than the recommended are used. The enzyme is active at pH 5.5-8.5.

WORKFLOW

1. Prepare ImpaRATOR

1.1 Reconstitute ImpaRATOR in 50 µl ddH_2O to 40 units/µl.

2. Add ImpaRATOR

2.1 Add 1 unit ImpaRATOR / 1 µg glycoprotein.[2]

3. Digestion

3.1 Incubate for 2 h[3] at 37°C.

2. A higher enzyme concentration may increase the digestion efficiency of individual glycoproteins. Optimization is then required.
3. Incubation times up to 18 hours may be required for complex substrates.

USA & Canada
Genovis Inc.
245 First Street, Suite 1800, Cambridge, MA 02142, USA
Phone: 1-855-782-0084 (toll free)
Fax: 1-858-524-3006

EMEA & Asia
Genovis AB
Box 790, SE-220 07 Lund, Sweden
Phone: +46 46 10 12 30
Fax: +46 46 12 80 20

support@genovis.com
www.genovis.com

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