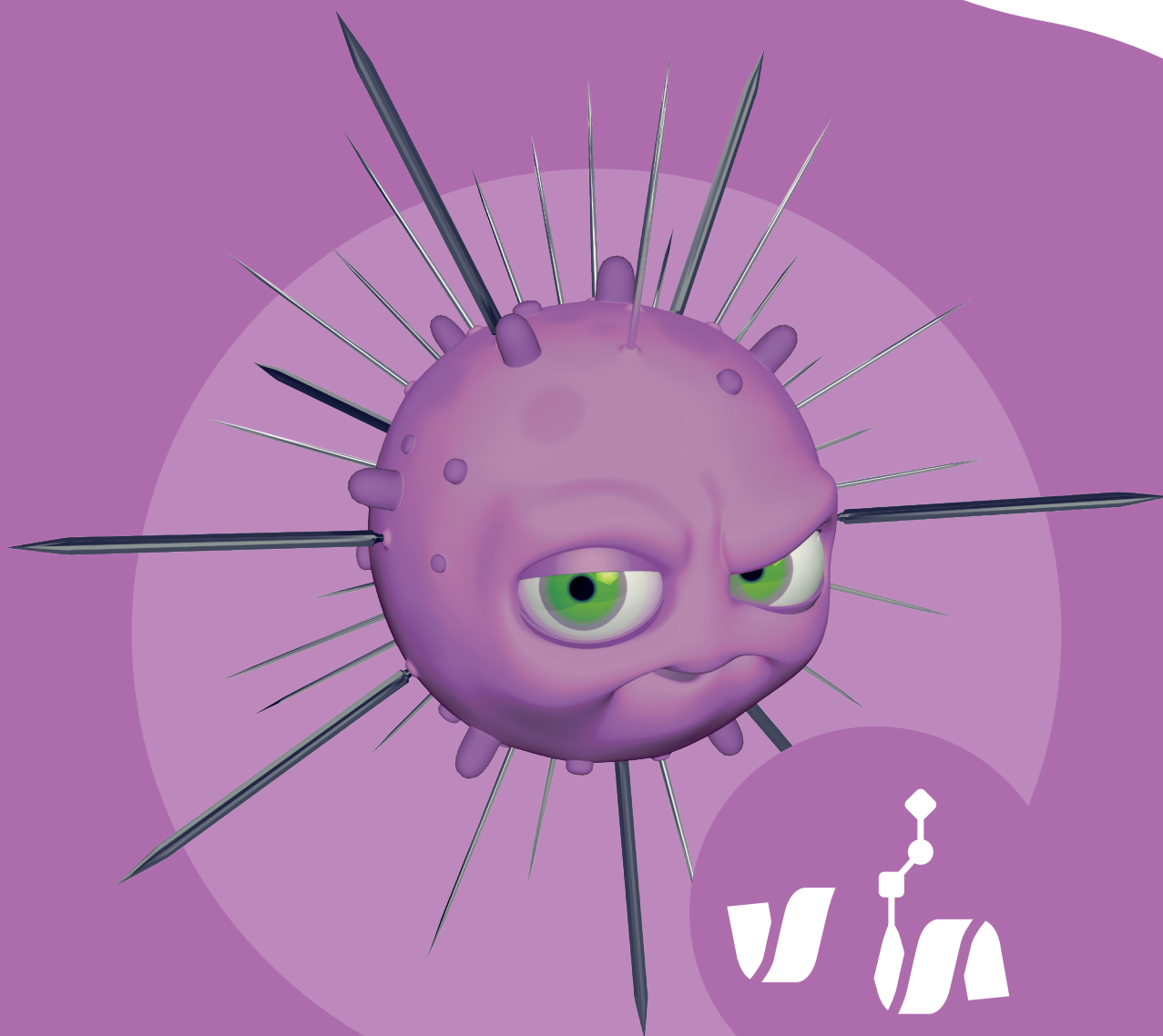


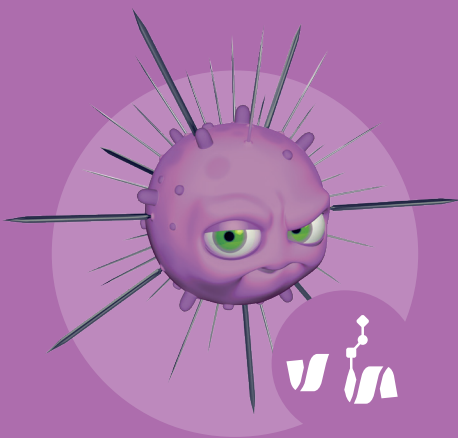


ImpaRATOR™

O-glycan-specific Protein Digestion

ImpaRATOR™

O-glycan-specific Protein Digestion



ImpaRATOR is an O-glycan-dependent protease that digests proteins carrying mucin-type O-glycans, including sialylated species, N-terminally of glycosylated Ser and Thr residues. The enzyme generates glycopeptides carrying O-glycans, which enables O-glycan profiling, site occupancy determination and O-glycopeptide mapping as well as middle-level approaches using LC-MS analysis.

-  Native proteins with mucin-type O-glycosylation, including sialylated species
-  N-terminally of O-glycosylated Ser and Thr
-  2h (complex substrates may require longer incubation times)
-  No reducing agents or co-factors needed

Digestion Workflow



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.

Product Formats



ImpaRATOR™ Lyophilized
Lyophilized enzyme for digestion of mucin-type O-glycoproteins and peptides, including sialylated O-glycan species

ImpaRATOR™ Lyophilized

PRODUCT	DESCRIPTION	ID
ImpaRATOR Lyophilized 2000 units	Process 2 mg O-glycoprotein	G1-IR1-020

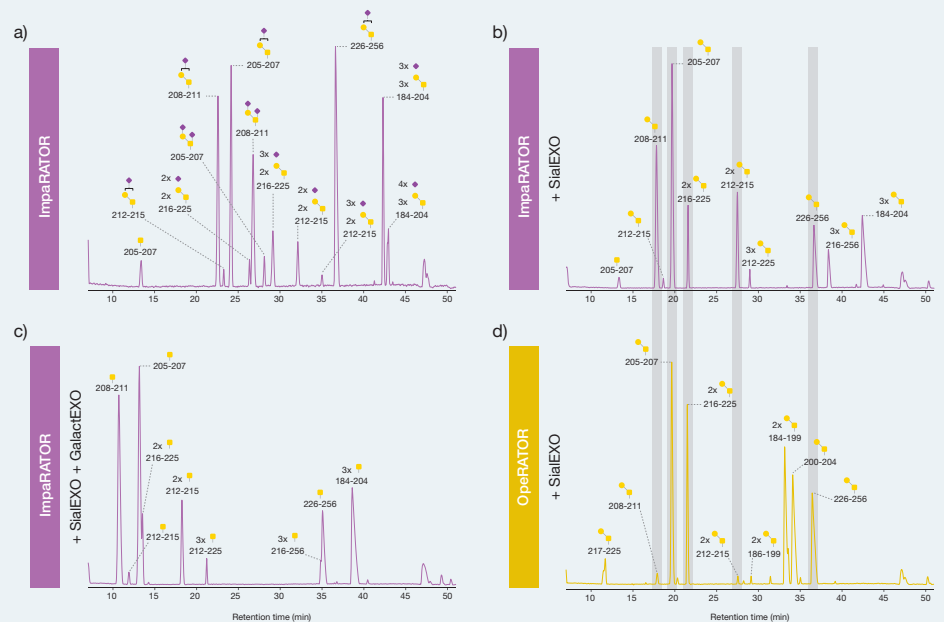


ImpaRATOR™ Accepts a Wide Range of O-glycan Species



To demonstrate the broad glycan specificity of ImpaRATOR, three samples of etanercept with different glycan compositions were treated with ImpaRATOR Lyophilized. The data demonstrates the acceptance of different glycan substrates of ImpaRATOR, and the ImpaRATOR-treated etanercept displayed a slightly different digestion pattern than the corresponding desialylated OperATOR-treated sample.

ImpaRATOR activity on etanercept with different glycan compositions. Etanercept **a)** untreated, **b)** treated with SialEXO, or **c)** treated with SialEXO and GalactEXO was digested by ImpaRATOR and FabRICATOR. **d)** Etanercept pretreated with SialEXO was digested with OperATOR and FabRICATOR. Peptides were reduced, alkylated and separated by HILIC (ACQUITY UPLC Glycoprotein Amide column, 300 Å, 1.7 µm, 2.1 mm × 150 mm) and analyzed on a Waters™ BioAccord™ LC-MS system. Shaded peaks indicate O-glycopeptides shared in (b) and (d).



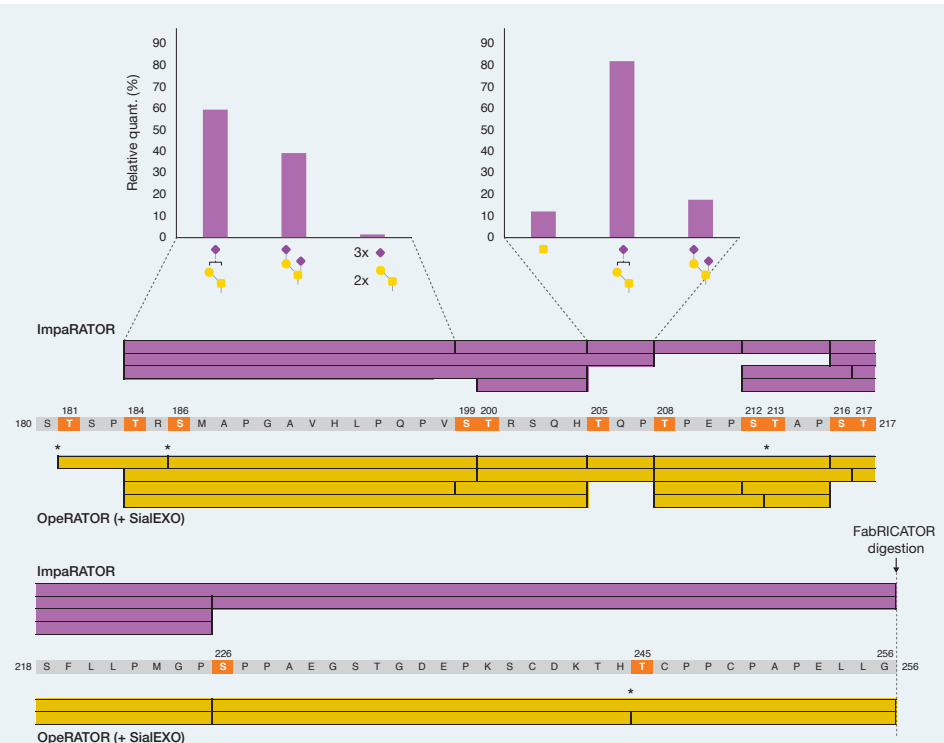
ImpaRATOR™ and OperATOR™ – Two Synergistic O-glycoproteases



The coverage of O-glycan sites in the heavily glycosylated region of etanercept was analyzed after digestion using ImpaRATOR or OperATOR (+ SialEXO). While OperATOR showed a higher O-glycan site coverage, digestion with ImpaRATOR enabled characterization of the O-glycan structures, including the sialylated species. Together, the ImpaRATOR and OperATOR enzymes provide complete information about both the glycan composition and O-glycan sites.

Sequence coverage of etanercept after digestion with ImpaRATOR or OperATOR.

Samples were prepared and data acquired as described in the figure above. Data was processed in UNIFI (Waters™) using a Peptide Map (Exact Mass MS) method. Graph inserts show the glycan distribution of the two example peptides T184-V198 and T205-P207 after digestion with ImpaRATOR. Sites cleaved only by OperATOR are indicated by asterisks.



EMEA & Asia – Genovis AB

Box 790, SE-220 07 Lund, Sweden

Phone: +46 46 10 12 30

Fax: +46 46 12 80 20

order@genovis.com

US & Canada – Genovis Inc.

245 First Street, Suite 1800, Cambridge, MA 02142, USA

Phone: 1-855-782-0084 (toll free)

Fax: 1-858-524-3006

orders.us@genovis.com

All rights reserved. Genovis products may be covered by one or more patents, trademarks and copyrights owned or controlled by Genovis AB. For more information about commercial

rights, please contact the Genovis team at licensing@genovis.com.

Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in

humans or animals. All goods and services are sold subject to Genovis' General Terms and Conditions of Sale.

© Genovis AB



GENOVIS

www.genovis.com