GingisREX®

FOR RESEARCH
USE ONLY
www.genovis.com

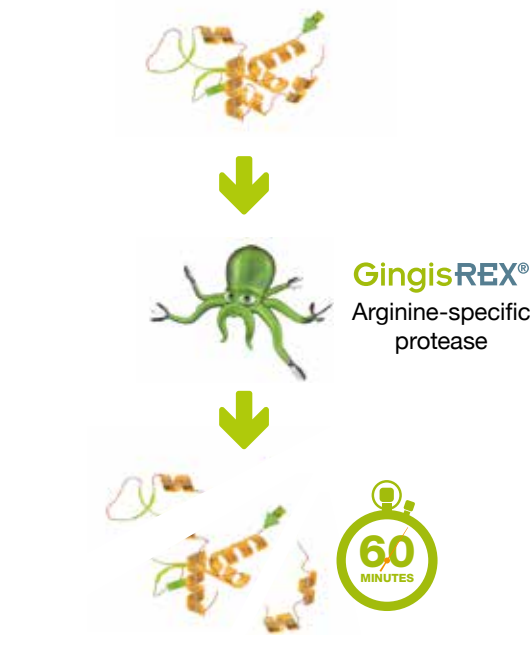
STORE AT
-20°C

SmartEnzymes™

GENOVIS

INSTRUCTIONS FOR PRODUCT

GingisREX® 5 µg (B0-GRX-005)



Last revised Feb 2020

PRODUCT DESCRIPTION

GingisREX (RgpB, Gingipain R) is a cysteine protease that specifically digests peptide bonds C-terminally of arginine residues, including sites next to proline. Longer peptides are generated compared to trypsin digestion, and with high resolution MS instruments, these peptides can be resolved, resulting in increased sequence coverage and identification of particular PTMs.

Cysteine is required for activity and specificity of the GingisREX enzyme. Cysteine is easily oxidized over time and it is important that the cysteine stays in its reduced form. TCEP can be added to the digestion sample to keep cysteine in its reduced form for a longer period. GingisREX is active in a broad pH range, 5.0-9.0, with optimal activity between pH 6.5-8.0. Digestion can be performed directly in high urea concentrations since GingisREX maintains high activity in up to 6 M urea. Buffers tested and compatible with GingisREX activity are Tris, Bis-Tris and ammonium bicarbonate.

Molecular weight: 48 kDa
Inhibitors: KYT-1, Iodoacetamide
Activator: Cysteine (> 10 mM)
Optimal pH: 6.5-8.0
Biological source: *Porphyromonas gingivalis*

Additional Materials Required

Enzyme activator: Cysteine
Reducing agent: TCEP (at neutral pH), or DTT
Denaturing agent: *Urea or SDS. Guanidin hydrochloride (Gd-HCl) should be avoided as the activity of GingisREX is substantially reduced in the presence of Gd-HCl.*
Digestion buffer: 0.1 M Tris, pH 7.4[1]

Preparations

1 Preparation of Cysteine

- Prepare cysteine and make sure that it is at neutral pH to not decrease the pH of the digestion buffer. The cysteine neutral solution must be freshly prepared and used the same day. Prepare a stock solution of 1 M cysteine in double distilled water (90 µl aliquots may be stored at -20 °C). To neutralize the cysteine solution, thaw one vial and add 10 µl 8 M NaOH to the 90 µl cysteine solution. This gives 100 µl of 0.9 M pH neutral cysteine solution ready to use. **Note! Use freshly prepared (within 6 h), it cannot be stored.**

2 Preparation of Urea

- 9 M urea: Dissolve 270 mg urea (MW 60.06 g / mol) in 260 µl digestion buffer – vortex vigorously and adjust the volume to 500 µl with digestion buffer. Use freshly prepared urea and if possible (depending on the protein to be digested), keep the temperatures in reactions with urea around 30°C to avoid carbamylation.

3 Preparation of Protein

- Dissolve the protein in the digestion buffer[1].

4 Preparation of GingisREX®

- Reconstitute GingisREX in 25 µL double distilled water to 0.2 mg / ml. Centrifuge the vial before addition of double distilled water and make sure that all lyophilized material is in the bottom of the vial and that it is dissolved.

In general, to obtain optimal digestion, proteins require efficient solubilization, denaturation and disulphide bond reduction (with subsequent alkylation). The following protocols are provided as a guideline to facilitate digestion with GingisREX. Optimization of the protocol might be necessary depending on the proteins to be digested.

Option 1. Digestion of Proteins in Solution – High Concentration of Urea

1 Solubilization/ Denaturation/ Disulphide Reduction

- In the chosen buffer, mix reagents to a final concentration of ~ 1 mg / ml protein, 4-6 M urea or 0.1% SDS for denaturation, 5 mM DTT or TCEP for reduction and 10 mM cysteine for GingisREX activation.

2 Add GingisREX®

- Add GingisREX in an enzyme:protein ratio of 1:200 to 1:20.

3 Digestion

- Incubate for 1-18 h at 30-37°C.

4 Alkylation

- Cool to room temperature (RT) and alkylate free sulfhydryls with 50 mM iodoacetamide (IAM) at RT for 30 min in the dark. This step will also inactivate GingisREX.

5 Quench IAM

- To avoid overalkylation, quench excess IAM with 20 mM DTT for 15 min at RT in the dark.

Option 2. Digestion of Protein in Solution

1 Solubilization/ Denaturation and Disulphide Reduction

- In the chosen buffer, mix reagents to a final concentration of ~ 4-6 mg / ml protein, 4-6 M urea or 0.1% SDS for denaturation, 5 mM DTT or TCEP for reduction.
- Incubate at 30-37°C for 30 min[2].

2 Alkylation

- Cool reaction to RT and add iodoacetamide (IAM) to a final concentration of 20 mM.
- Incubate in the dark at RT for 30 min.

3 Quench IAM

- Add cysteine to a final concentration of 100 mM. To quench excess of IAM and activate GingisREX, incubate at RT for 15 min.

4 Sample Dilution

- Dilute the sample with digestion buffer 4-5 X to decrease the urea concentration (less carbamylation). Add TCEP[3] to a final concentration of 5 mM.

5 Add GingisREX®

- Add GingisREX in an enzyme: protein ratio of 1:200 to 1:20.

6 Digestion

- Incubate for 1-18 h at 30-37°C.
- The reaction can be stopped by addition of trifluoroacetic acid or formic acid to a final concentration of 0.5-1%.

Quality Control

GingisREX is tested to ensure lot-to-lot consistency.

The activity and specificity of GingisREX is tested on oxidized insulin β-chain at a protease: substrate ratio of 1:20 and 1:200. The digestion reaction is performed at 37 °C for 30 min and 18 h and analyzed with RP-HPLC. Two peaks specific for GingisREX activity are generated.

Notes

1. *A digestion buffer between pH 5.5-9.0 may be used. Optimal pH is at 6.5-8.0 and with lower pH the incubation time and/or enzyme: protein ratio needs to be increased.*
2. *Depending on protein, heating may be required to solubilize and denature the protein.*
3. *This is to keep the cysteine in its reduced form for activation of GingisREX. If TCEP is added in the reduction step, this step will not be necessary as TCEP unlike DTT is not inactivated by IAM.*

GingisREX®

Legal and Disclaimers

For more information about commercial rights, please contact the Genovis team at info@genovis.com.

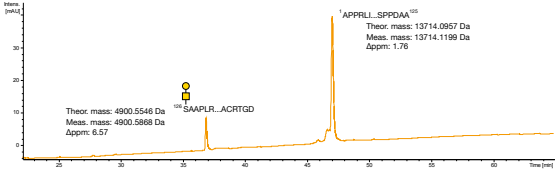
Genovis products are intended for research use only. They are not intended to be used for therapeutic or diagnostic purposes in humans or animals.

OpeRATOR®

O-glycan-specific Endoprotease

OpeRATOR is a novel tool for analysis of mucin-type O-glycans on glycoproteins. The protein binds to O-glycans and digests the peptide backbone N-terminally of the S/T glycosylation sites.

- O-glycan-specific, mucin-type
- Requires O-glycans for activity
- Generates glycopeptides with O-glycans and allows for O-glycan profiling and site occupancy determination using mass spectrometry



Erythropoietin (EPO) is a ~30 kDa glycoprotein with one core 1 O-glycan site. The protein was used here as a substrate to demonstrate the specific activity of the OpeRATOR protease. OpeRATOR hydrolyzed the protein N- terminally of the serine O-glycan site, and after reduction of disulfide bridges, the resulting two fragments were separated and intact mass was analyzed by Q-TOF MS using ESI.

US & Canada

Genovis Inc.
245 First Street, Suite 1800
Cambridge, MA 02142
USA

Customer service: 617-444-8421
Order phone (toll free): 855-782-0084
Order fax: 858-524-3006
Email: orders.us@genovis.com

EMEA & Asia

Genovis AB
Box 790
SE-220 07 Lund
Sweden

Customer service: +46 46 10 12 30
Order phone: +46 46 10 12 30
Order fax: +46 46 12 80 20
Email: order@genovis.com