GingisREX®

INSTRUCTIONS

Version 17.1.1

Instructions for Product
GingisREX® (B0-GRX-005) 5 µg

Content and Storage

GingisREX is formulated in 20 mM Bis-Tris, 150 mM NaCl, 5 mM $CaCl_2$, pH 6.8 without preservatives, and supplied as a lyophilized powder.
GingisREX is shipped on ice. Vials should be stored at -20°C upon arrival.
After reconstitution GingisREX is stable for at least 1 month at +4-8°C.
GingisREX is for R&D use only.

Application

GingisREX protease is for digestion of proteins prior to LC or LC-MS.

Product Description

GingisREX (Rgp, Gingipain R) is a cysteine protease that specifically digests peptide bonds C-terminal to arginine residues, including sites next to proline. Longer peptides are generated, compared to trypsin digestion, and with the application of high resolution LC and MS instruments, these can be resolved, resulting in increased sequence coverage and identification of particular PTMs.
Cysteine is required for activity and specificity of GingisREX enzyme. Cysteine is easily oxidized over time and it is important that the cysteine maintains its reducing activity (i.e. to prevent that cysteine gets oxidized). TCEP can be added to the digestion sample to maintain cysteine in its reduced form for a longer period of time. 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 6M 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 (>10mM)
Optimal pH: 6.5-8.0
Biological source: *Porphyromonas gingivalis*
Sequence: Acc no. U85038

Additional Materials Required

Enzyme activator: Cysteine
Reducing agent: TCEP (at neutral pH), Cysteine (at neutral pH) or DTT
Denaturing agent: Urea or SDS
Digestion buffer: 0.1M Tris, pH 7.4[1]

Preparations

Preparation of cysteine

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

Preparation of urea

- 9M urea: Dissolve 270mg urea (MW 60.06 g/mol) in 240µl digestion buffer – vortex vigorously and adjust volume to 500µl with digestion buffer.

Preparation of protein

- Dissolve protein in digestion buffer[1].

Preparation of GingisREX®

- Reconstitute GingisREX in 25µL double distilled water. Centrifuge the vial before addition of double distilled water and make sure all lyophilized material is in the bottom of the vial and is dissolved.

Protocol

In general, to obtain optimal protein digestion and good sequence coverage, 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.

Option 1.
Digestion of protein in solution (one pot reaction)

- Mix 1mg/ml protein to be digested, 4M urea, 5mM TCEP and 10mM cysteine. Add digestion buffer to final volume.
- Add GingisREX in an enzyme:protein ratio of 1:20 to 1:200.
- Incubate at 37°C for 1-18h.
- Stop the reaction with 50-100mM iodoacetamide. Incubate in the dark for at least 30 min at room temperature.

Option 2.
Digestion of protein in solution (after denaturation, reduction and alkylation)

- **Solubilization/Denaturation**: Dissolve protein in a denaturing reagent such as 4-8M urea or 0.1% SDS.
- **Disulphide Reduction:** Add reducing agent, 5 mM TCEP or DTT and incubate at room temperature for 30 min[2].
- **Alkylation**: Add iodoacetamide to a final concentration of 10 mM and incubate in the dark at room temperature for 30 min.
- **Digestion**
    - Add 20 mM cysteine.
    - Add 10 mM TCEP[3].
    - Add digestion buffer to final volume.
    - Add GingisREX in an enzyme:protein ratio of 1:20 to 1:200.
    - Incubate at 37°C for 1-18h.
    - The reaction may be stopped with trifluoroacetic acid or formic acid to a final concentration of 0.5-1%.

Notes

1. 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 needs to be increased and/or enzyme:protein ratio increased.
2. Depending on protein, heating may be required to solubilize and denature the protein.
3. This is to make sure that the cysteine is kept in its reduced form. If TCEP is added in the reduction step, this step will not be necessary.

Quality Control

GingisREX is tested to ensure lot-to-lot consistency.
Activity (performance) and purity is checked with HPLC.
The 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 18h and analyzed with RP- HPLC. Two peaks specific for GingisREX™ activity are generated.

GingisREX®

Customer Service USA Phone: 617-444-8421 | info@genovis.com | Genovis AB, 245 First Street, suite 1800, Cambridge, MA 02142, USA **Customer Service Europe** Phone: 0046 (0)46 10 12 30 | info@genovis.com | Genovis AB, Box 790, SE-220 07 Lund, Sweden **Support** support@genovis.com **Order** order@genovis.com | Fax: 0046 (0)46 12 80 20
www.genovis.com