SmartEnzymes™

GENOVIS

GingisREX®

Lyophilized

STORE AT

-20°C

FOR RESEARCH USE ONLY

Instructions for Use

GingisREX® Lyophilized 5 µg (B0-GRX-005)
Process 0.1-1 mg protein

DOWNLOAD INSTRUCTIONS FOR USE

www.genovis.com/ifu-B0-GRX

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Lyophilized Enzyme for Arginine-specific Protein Digestion

GingisREX (RgpB) is an arginine-specific protease that digests proteins C-terminally of arginine residues, including Arg-Pro linkages that are difficult to digest with other enzymes.

GingisREX Lyophilized is active at a broad pH range of 5.0-9.0, with optimal activity at pH 6.5-8.0. L-cysteine is required for the activity and specificity of the enzyme.

The GingisREX enzyme is purified from *Porphyromonas gingivalis*. It has a molecular weight of 48 kDa.

CONTENT AND STORAGE

GingisREX Lyophilized is supplied lyophilized in 20 mM Bis-Tris, 150 mM NaCl, 5 mM $CaCl_2$, pH 6.8, with no preservatives added.

GingisREX Lyophilized is shipped cold, and should be stored at -20°C upon arrival. After reconstitution, GingisREX Lyophilized is stable for at least 1 month at +4-8°C.

GingisREX Lyophilized is for R&D use only.

QUALITY CONTROL

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

The activity and specificity of GingisREX Lyophilized 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 minutes and 18 hours, and analyzed with RP-HPLC.

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FabRICATOR®
Below hinge digestion of IgG

FabALACTICA®
Above hinge digestion of human IgG1

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Hydrolysis of flexible linkers

Preparations

Important Information

- The L-cysteine solution must be prepared and used the same day (within 6 h), it cannot be stored.
- In general, to obtain optimal digestion, proteins require efficient solubilization, denaturation and disulphide bond reduction (with subsequent alkylation). The two protocols in this instruction are provided as guidelines to facilitate digestion with GingisREX. Optimization of the protocol might be necessary depending on the substrate.

Additional Materials Required

- L-cysteine hydrochloride monohydrate.
- Reducing agent (TCEP at neutral pH, or DTT)
- Denaturing agent (Urea or SDS). Guanidine hydrochloride (Gd-HCl) should be avoided since it inhibits the activity of GingisREX.
- Reaction buffer: 0.1 M Tris, pH 7.4[1].

Preparation of L-cysteine

Prepare L-cysteine and make sure that the pH is neutral, to not decrease the pH of the reaction buffer. Prepare a stock solution of 1 M L-cysteine hydrochloride in ddH$_2$O. We recommend that you store 90 µl aliquots at -20°C. To neutralize the L-cysteine solution, thaw one 90 µl vial and add 10 µl 8 M NaOH. This gives 100 µl ready-to-use 0.9 M L-cysteine solution, with neutral pH.

Preparation of 9 M Urea

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

Preparation of GingisREX

Centrifuge the GingisREX Lyophilized vial and make sure that all lyophilized material is in the bottom of the vial. Reconstitute the enzyme by adding 25 µl ddH$_2$O to a concentration of 0.2 mg/ml. Make sure that all lyophilized material is dissolved.

1. A reaction 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.

Arginine-specific Protein Digestion

Sample Preparation
Dissolve the protein in the reaction buffer.[1]

Protocol A: Digestion of Protein in Solution – High Concentration of Urea

A1. Solubilization/Denaturation/Disulphide Reduction

A1.1 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.
- 10 mM cysteine for GingisREX activation.

A2. Add GingisREX

A2.1 Add GingisREX in an enzyme:protein ratio of 1:200 to 1:20.

A3. Enzymatic Reaction

A3.1 Incubate for 1-18 h at 30-37°C.

A4. Alkylation

A4.1 Cool down to room temperature (RT) and alkylate free sulfhydryls with 50 mM iodoacetamide for 30 min at RT in the dark. This step will also inactivate GingisREX.

A5. Quench Iodoacetamide

A5.1 To avoid overalkylation, quench excess iodoacetamide with 20 mM DTT for 15 min at RT in the dark.

Protocol B: Digestion of Protein in Solution

B1. Solubilization/Denaturation/Disulphide Reduction

B1.1 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.

B1.2 Incubate for 30 min at 30-37°C.[2]

B2. Alkylation

B2.1 Cool down reaction to RT and add iodoacetamide to a final concentration of 20 mM.

B2.2 Incubate for 30 min at RT in the dark.

B3. Quench Iodoacetamide

B3.1 Add cysteine to a final concentration of 100 mM. To quench excess of iodoacetamide and activate GingisREX, incubate for 15 min at RT.

B4. Sample Dilution

B4.1 Dilute the sample with reaction buffer 4-5× to decrease the urea concentration (less carbamylation). Add TCEP[3] to a final concentration of 5 mM.

B5. Add GingisREX

B5.1 Add GingisREX in an enzyme:protein ratio of 1:200 to 1:20.

B6. Enzymatic Reaction

B6.1 Incubate for 1-18 h at 30-37°C.

B6.2 The reaction can be stopped by addition of trifluoroacetic acid or formic acid to a final concentration of 0.5-1%.

2. Depending on protein, heating may be required to solubilize and denature the protein prior to the incubation.
3. This step 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 iodoacetamide.

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