



(N)-S-T-R | E-G-(C)

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

GingisREX™ Lyophilized 5 × 5 µg (B0-GRX-025)

Process 5 × 0.1-1 mg protein



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 7.4¹.

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 ddH₂O. 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. This protocol is scalable. If more than 500 µl 9 M urea is required.

Preparation of 1 × 5 µg GingisREX Lyophilized

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 ddH₂O 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:

- ~4-6mg/ml protein.
- 4-6M urea or 0.1% SDS for denaturation.
- 5mM 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 20mM.

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

B3. Quench Iodoacetamide

B3.1 Add cysteine to a final concentration of 100mM. 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 4-5x to decrease the urea concentration (less carbamylation). Add TCEP³ to a final concentration of 5mM.

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 1-18h 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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