



FabRICATOR® Xtra

LALA Lyophilized

STORE AT

-20°C



FOR RESEARCH USE ONLY

Instructions for Use

FabRICATOR® Xtra LALA Lyophilized 2000 units
(A0-XA1-020)

Process 2 mg human IgG1-LALA



Preparations

Additional Materials Required

- Reaction buffer: PBS (10mM sodium phosphate, 150mM NaCl), pH 7.4.¹

1. PBS including 2.7mM KCl can also be used. Optimization may be required if buffers other than the recommended are used. The enzyme is active at pH 7.0- 9.0 and at salt concentrations below 200mM.

Below Hinge Digestion of Hinge-mutated IgG, Including LALA

Sample Preparation

Prepare the IgG in the reaction buffer. The final IgG concentration in the reaction should be 0.5-5 mg/ml.²

1. Prepare FabRICATOR Xtra LALA

1.1 Reconstitute FabRICATOR Xtra LALA in 80 µl ddH₂O to a concentration of 25 units/µl.

2. Add FabRICATOR Xtra LALA

2.1 Add FabRICATOR Xtra LALA according to the reaction conditions described in Table 1.

2.2 *Optional: add a reducing agent (e.g. up to 20 mM DTT) to generate reduced IgG subunits in a single step³.*

3. Enzymatic Reaction

3.1 Incubate at 37°C according to the reaction conditions described in Table 1.

Table 1. Recommended Reaction Conditions and Digestion Sites for Different Hinge-mutated IgG Formats⁴

Antibody	Enzyme Amount	Reaction Time	Digestion Site(s)
hIgG1-LALA (L234A, L235A)	1 unit / µg IgG	1 h	Primary: CPAPE AAG / GPSVF Secondary: CPAPE AA / GGPSVF
hIgG4-FALA (F234A, L235A)	1 unit / µg IgG	1 h	Primary: CPAPE AA / GGPSVF Secondary: CPAPE AAG / GPSVF
hIgG1-LAGA (L235A, G237A)	2 units / µg IgG	2 h	Primary: CPAPE LA / G A PSVF Secondary: CPAPE LAG / A PSVF
hIgG1-FES (L234F, L235E, P331S)	3 units / µg IgG	O/N	CPAPE FEG / GPSVF

- Using lower substrate concentrations may negatively impact enzyme performance.
- Adding a reducing agent slightly increases enzymatic activity of FabRICATOR Xtra LALA.
- Other reaction conditions may be required for IgG with different/additional mutations. Apart from the lower hinge, the regions around amino acid residues 265-269, 296-298 and 322-333 in the IgG CH2 domain have been shown to be involved in the enzyme's interaction with the IgG substrate. Mutations in these regions may negatively affect or completely inhibit digestion with FabRICATOR Xtra LALA. Optimization is then required.

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