

FabRICATOR®

Instructions for product no

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|------------|-------------|------------------------------|
| A0-FR1-005 | 500 units | Cleavage of up to 0.5mg IgG |
| A0-FR1-010 | 1000 units | Cleavage of up to 1.0mg IgG |
| A0-FR1-020 | 2000 units | Cleavage of up to 2.0mg IgG |
| A0-FR1-050 | 5000 units | Cleavage of up to 5.0mg IgG |
| A0-FR1-250 | 25000 units | Cleavage of up to 25.0mg IgG |

Product Description

FabRICATOR® is an enzyme for preparation of F(ab')₂. FabRICATOR® is a digestive enzyme that cleaves IgG only at one specific site below the hinge region resulting in F(ab')₂ and Fc-fragments. Since FabRICATOR® only cleaves at one specific site below the hinge region, there is no risk of over digestion if the incubation time is increased for several hours.

FabRICATOR® cleaves all subclasses of human, monkey, rabbit and sheep IgG but only subclass IgG2a and IgG3 of mouse IgG. Cleavage of mouse IgG2a and IgG3 requires a special protocol with increased FabRICATOR® concentration and prolonged incubation time.

Best activity is obtained at pH 6.6 and 37°C. It is possible to use a buffer with a higher pH and increasing the reaction time. Digestion can also be done at room temperature with prolonged incubation time. Optimization is required.

FabRICATOR® contains a His-tag and can therefore easily be removed after digestion.

Content and storage

FabRICATOR® is supplied as lyophilized powder from 50 mM sodium phosphate, 150 mM NaCl, pH 6.6, with no preservatives added.

FabRICATOR® is shipped on ice. It should be stored at -20°C upon arrival.

After reconstitution FabRICATOR® is stable for 1 month at +4-8°C.

FabRICATOR® is for R&D use only.

Unit Definition

One unit cleaves ≥ 95% of 1µg human IgG when incubated in 50mM sodium phosphate pH 6.6, 150mM NaCl at 37°C for 30min.

Quality Control

FabRICATOR® is tested to ensure lot-to-lot consistency.

FabRICATOR® is tested for absence of microbial contamination with blood agar plates, Sabaraud dextrose agar plates and fluid thioglycolate medium.

Additional Materials Required

- **Cleavage Buffer:** 50mM sodium phosphate, 150mM NaCl, pH 6.6
- Test tubes

Method

Cleavage of IgG

- Make sure your antibody is in cleavage buffer (See Additional Material Required above) with a concentration of 0.5-10mg/ml.
- Reconstitute FabRICATOR® in double distilled H₂O according to table 1 below. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
- Add FabRICATOR® to the IgG. Add 1 unit FabRICATOR®/1µg IgG. *For cleavage of mouse IgG2a and IgG3 a special protocol is required, see below.
- Incubate for 30 minutes in cleavage buffer at 37°C. *For cleavage of mouse IgG2a and IgG3 a special protocol is required, see below.

| Product | Product Size | Reconstitution volume | Concentration | Volume for 1000 units |
|------------|--------------|-----------------------|---------------|-----------------------|
| A0-FR1-005 | 500 units | 50µL | 10 units/µL | --- |
| A0-FR1-010 | 1000 units | 50µL | 20 units/µL | 50µL |
| A0-FR1-020 | 2000 units | 30µL | 67 units/µL | 15µL |
| A0-FR1-050 | 5000 units | 75µL | 67 units/µL | 15µL |

Table 1.

*Cleavage of mouse IgG2a and IgG3

- Make sure your antibody is in cleavage buffer (See Additional Material Required above) with a concentration of 0.5-10mg/ml.
- Reconstitute FabRICATOR® in double distilled H₂O according to table 1 above. To prevent microbial contamination, sodium azide can be added to the solution to a final concentration of 0.02 - 0.05% (w/v).
- Add FabRICATOR® to the mouse IgG. Add 5 units FabRICATOR®/1µg mouse IgG.
- Incubate for 2-3 hours in cleavage buffer at 37°C. Optimization is required for your specific antibody.

Product References

Mary H. Ryana, Diane Petrone, Jennifer F. Nemetha, Evan Barnathan, Lars Björck, Robert E. Jordan: *Proteolysis of purified IgGs by human and bacterial enzymes in vitro and the detection of specific proteolytic fragments of endogenous IgG in rheumatoid synovial fluid*, Molecular Immunology, October 2007.

Application note

FabRICATOR - perfect F(ab')₂ fragments in minutes