INSTRUCTIONS **Last revised September 2018**

Instructions for product no:

A0-FRC-050 FabRICATOR-HPLC Column

Content and Storage

The FabRICATOR-HPLC column is packed with FabRICATOR enzyme immobilized on an HPLC compatible resin in 10 mM sodium phosphate, 140 mM NaCl, pH 7.4 with 0.02% sodium azide. Packed column (2.1 mmD/50 mmL) with sealing end caps.

The FabRICATOR-HPLC column is shipped cold and should be stored at +4-8°C.

Store the column at +4-8°C in 10 mM sodium phosphate, 140 mM NaCl, pH 7.4 when not in use.
Store the column with the endcaps in place, carefully sealed to prevent drying.
For long-term storage of the column 0.02% (w/v) sodium azide can be added as a preservative.

Product Description

FabRICATOR (IdeS) is an enzyme that digests IgG at one specific site just below the hinge region, resulting in F(ab')2 and Fc fragments with no risk of over-digestion.

FabRICATOR digests all subclasses of human, monkey, rabbit and sheep IgG but has limited activity on mouse IgG2a and mouse IgG3. FabRICATOR digests IgG under physiological reaction conditions, thus preserving the immunoreactivity. FabRICATOR is active in most buffers at neutral pH and is irreversibly inactivated at pH < 5. The FabRICATOR enzyme is produced recombinantly in *E. coli*.

FabRICATOR-HPLC columns are packed with POROS™* resin, cross-linked poly(styrene-divenylbenzene) beads with FabRICATOR enzyme covalently immobilized to its surface.
The column has the dimensions 2.1 mmD/50 mmL.

The FabRICATOR-HPLC column is designed to be an analytical tool for rapid on-column digestion in workflows for characterization of antibodies and Fc-fusion proteins. After digestion, the fragments can be analysed using reversed phase HPLC, mass spectrometry or other analytical method.

FabRICATOR-HPLC is for R&D use only.

Additional Materials Required

20 mM DTT for activation of the column.
Digestion buffer at neutral pH, for example PBS.

General considerations

Different antibodies and Fc-fusion proteins are digested at different rates. To enhance the digestion efficiency, it is recommended to use a column temperature of 37°C and decrease the flow rate. As a guidance, flow rates over the column of 25 to 50 µl/min at a temperature of 37°C usually give a good digestion. Injections with DTT before adding the antibody sample is important to activate the column, and could be beneficial if a decrease in digestion efficiency is observed over time.

*This product is provided under an intellectual property license from Life Technologies Corporation, see following page.

Performance

The performance of the FabRICATOR-HPLC column at 37°C is typically >200 injections with a digestion efficiency of >95% for 10 µg human IgG1 (1 mg/ml) sample in PBS, pH 7.4.

Protocol

Preparation of IgG

Dilute the IgG in the digestion buffer used to equilibrate the column to a concentration appropriate for the specific application.

Antibody Subunit Generation

1. Initial column equilibration is achieved by washing with 10 column volumes of the digestion buffer of choice at a flow rate of 100 µl/min.
2. Activate the column with three 20 µl injections of 20 mM DTT at a flow rate of 50 µl/min.
3. Equilibrate the column with digestion buffer at a flow rate of 100 µl/min for at least 60 min.
4. Inject the antibody sample at a flow rate of 25 to 50 µl/min and elute the antibody from the column with 2- 3 column volumes.
5. Collect the processed material from the column for subsequent analysis.
6. Continue washing with 3-4 column volumes of digestion buffer.
7. Inject a new sample, repeat from step 4.

The digestion efficiency of the antibody can be controlled by adjusting the flow rate. A residence time of less than five minutes on the column is usually sufficient for digestion.

After digestion, the fragments can be analyzed using reversed-phase HPLC, mass spectrometry, or other analytical method.

When all samples have been processed, wash the column with digestion buffer and store it, as described in section Content and Storage.