

EK-Away™ Resin

Catalog Numbers

R18001
R18002

Quantity

7.5 mL of resin
4 × 7.5 mL of resin

Store resin at 4°C

Description

EK-Away™ Resin is used to remove EnterokinaseMax™ (or other enterokinases) after digestion of fusion protein containing the enterokinase cleavage sequence. The resin consists of soybean trypsin inhibitor immobilized on 4% beaded agarose. Using 150 µL of EK-Away™ slurry, 99% of the EKMax™ Enterokinase used in a 500-µL digestion containing 5 units of EKMax™ Enterokinase can be removed in 15 minutes.

Caution

Avoid skin contact and inhalation of EK-Away™ Resin and Buffers. Wear gloves when handling reagents and follow safe laboratory procedures.

Contents and Storage

EK-Away™ Resin is available in two sizes. Catalog no. R18001 contains 7.5 mL of resin, and is capable of binding 250 units of EKMax™ Enterokinase. Catalog no. R18002 contains 4 × 7.5 mL of resin, and is capable of binding 1000 units of EKMax™ Enterokinase.

| Item | Composition | Catalog no. | |
|-------------------------------|--|-------------|------------|
| | | R18001 | R18002 |
| EK-Away™ Resin | 50% (v/v) slurry in 0.5 M NaCl, 0.02% Thimerosal | 7.5 mL | 4 × 7.5 mL |
| 10X EK-Away™ Stripping Buffer | 1 M NaCl 1 M Formic Acid, pH 3 | 4 mL | 4 × 4 mL |
| 10X EK-Away™ Binding Buffer | 5 M NaCl 0.5 M Potassium Phosphate, pH 8 | 5 mL | 4 × 5 mL |

EK-Away™ Resin is shipped at room temperature. **DO NOT FREEZE EK-Away™ Resin.**

| Item | Storage |
|-------------------------------|------------------|
| EK-Away™ Resin | 4°C |
| 10X EK-Away™ Stripping Buffer | Room Temperature |
| 10X EK-Away™ Binding Buffer | Room Temperature |

Specifications

1 unit* of EKMax™ Enterokinase is defined as the amount of EKMax™ Enterokinase that digests 20 µg of a thioredoxin-chloramphenicol acetyl transferase fusion protein to 90% completion in 16 hours at 37°C in 50 mM Tris-HCl, pH 8.0, 1 mM CaCl₂, and 0.1% Tween-20.

* 1 Invitrogen unit of EKMax™ Enterokinase is equivalent to ~190 trypsinogen activation units.

Certificate of Analysis

The Certificate of Analysis provides detailed quality control and product qualification information for each product. Certificates of Analysis are available on our website. Go to www.invitrogen.com/support and search for the Certificate of Analysis by product lot number, which is printed on the box.

Intended Use

For research use only. Not intended for human or animal diagnostic or therapeutic uses.



Before Starting

- Determine the amount of EK-Away™ slurry needed to remove EKMax™ Enterokinase from the digestion. The amount of EK-Away™ slurry = $30 \mu\text{L} \times N$ units, where N = the number of EKMax™ Enterokinase units in the digestion.
- Dilute the 10X EK-Away™ Stripping Buffer and the 10X EK-Away™ Binding Buffer to 1X solutions by adding 10 μL of the 10X solution to 90 μL of sterile water for every 100 μL of 1X solution needed. Refer to the **Instructions for Use (steps 1–7)** to determine the amount of each 1X solution needed.

Example: If your digestion contains 5 units of EKMax™ Enterokinase, you will need to use 150 μL of EK-Away™ slurry. 150 μL of EK-Away™ slurry will yield a bed volume of 75 μL of EK-Away™ resin. You will need $2 \times 300 \mu\text{L}$ of each 1X solution to wash the resin (**Instructions for Use, steps 1–6**) and 75 μL of 1X EK-Away™ Binding Buffer to resuspend the resin (**Instructions for Use, step 7**).

Instructions for Use

1. Resuspend the EK-Away™ Resin in 4 times the bed volume with 1X EK-Away™ Stripping Buffer. Rock the sample for 2 minutes.
2. Pellet the resin by centrifuging for 1 minute at $800 \times g$. Remove and discard the supernatant.
3. Repeat steps 1 and 2.
4. Resuspend the EK-Away™ Resin in 4 times the bed volume with 1X EK-Away™ Binding Buffer. Rock the sample for 2 minutes.
5. Pellet resin by centrifuging for 1 minute at $800 \times g$. Remove and discard the supernatant.
6. Repeat steps 4 and 5.
7. Resuspend the EK-Away™ Resin to make a 50% slurry in 1X EK-Away™ Binding Buffer.
8. Add 0.1 volume of 10X EK-Away™ Binding Buffer to the EKMax™ Enterokinase digestion.

Example: If the EKMax™ Enterokinase digestion is 30 μL , add 3 μL of 10X EK-Away™ Binding Buffer.

9. Add the prewashed EK-Away™ Resin to the EKMax™ Enterokinase digestion. Keep the EK-Away™ Resin suspended in the digestion mix for 15 minutes by gently mixing, or slowly rocking the tube.
10. Centrifuge the resin for 2 minutes at $5000 \times g$ and save the supernatant. The supernatant contains your protein and can be analyzed by an activity assay or by SDS-PAGE.

Note: Your protein will be in a 0.5 M NaCl, 0.05 M KPO_4 solution.

11. **Optional:** You may wish to do an additional wash with 2 times the bed volume of 1X EK-Away™ Binding Buffer and save the supernatant to ensure that you have recovered all of your protein from the EK-Away™ Resin.

References

Grant, D. A. W. and Hermon-Taylor, J. (1977) Hydrolysis of Artificial Substrates by Enterokinase and Trypsin and the Development of a Sensitive Specific Assay for Enterokinase in Serum. *Biochem J.* **155**: 243-254.

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