

ab286883 – HRV14 3C (PreScission) Protease Agarose Beads

For the efficient and convenient cleavage of recombinant fusion proteins containing PreScission cleavage sequence (Leu-Glu-Val-Leu-Phe-Gln/Gly-Pro).
For research use only - not intended for diagnostic use.

For overview, typical data and additional information please visit:

<http://www.abcam.com/ab286883>

Storage and Stability

On receipt product should be stored at -20°C.

Materials Supplied

HRV14 3C (PreScission) Protease Agarose Beads Supplied as 50% slurry in 100% glycerol

General Protocol

The target fusion protein should be purified to homogeneity and dialyzed against 50 mM Tris buffer, 0.1 M NaCl, 10 mM DTT, pH 8.0 before setting up the cleavage reaction. In order to find the optimum cleavage condition for a target fusion protein, it is recommended to run preliminary cleavage reactions at a small scale. Successful cleavage with HRV14 3C Protease is dependent upon proper folding of the fusion protein that enables access of the PreScission cleavage site by the enzyme. Once the optimum cleavage condition is obtained, the reaction can be scaled up to cleave the entire amount of the target fusion protein.

Cleavage Protocol

1. Re-suspend the beads by gentle swirling. Do not Vortex.
2. Aliquot 50 µl of the suspended slurry and add to 100 µg of the fusion protein (0.25-1 mg/ml) in an Eppendorf tube.
3. Mix gently by inverting the tube (do not vortex) and gently shake on a rotary shaker at room temperature. At regular time intervals spin down the tube to aliquot a test sample and freeze it immediately. At the end of the reaction, analyze the samples by SDS-PAGE.
4. After the fusion protein is completely cleaved, spin down the reaction mixture for 2-3 min at 5000 rpm. Remove the supernatant and wash the beads with 0.1 ml of 50 mM Tris buffer, pH 8. Further chromatography may be necessary to remove the cleaved fragments from the target protein.

Technical Support

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