EZCut™ HRV14 3C (Precission) Protease Agarose Beads

CATALOG #:	M1029-1	1 ml
	M1029-5	5 ml
	M1029-25	25 ml

LIGAND DENSITY: 100 µg/ml of the resin.

FORMULATION: Provided as 50% slurry in 100% glycerol.

STORAGE CONDITIONS: Store at -20°C.

DESCRIPTION: Active HRV14 3C Protease (Precission Protease) domain from human rhinovirus type 14 has high selectivity and site-specificity for the Precission cleavage site (Leu-Glu-Val-Leu-Phe-**Gln/Gly**-Pro), thereby allowing the production of recombinant proteins with minimal number of extra amino acids. BioVision's EZCut™ HRV14 3C (Precission) Protease Agarose is prepared by covalent coupling of active HRV14 3C Protease to activated 4% cross-linked agarose beads. EZCut™ HRV14 3C (Precission) Protease Agarose Beads are designed for efficient cleavage of recombinant fusion proteins, containing the Precission protease cleavage site, circumventing the need for chromatographic techniques to remove the protease after cleavage reaction is completed. The optimal temperature for cleavage is 30°C; however, the enzyme is active over wide ranges of temperature. 25 µl of beads (or 50 µl of the 50% slurry) are sufficient to cleave >80% of 100 µg control SUMO-GFP protein, containing Precission cleavage site, at room temperature in 24 h. These beads can be regenerated for repeat use and the reusability depends on the usage and handling of the product.

APPLICATIONS: Efficient and convenient cleavage of recombinant fusion proteins containing Precission cleavage sequence (Leu-Glu-Val-Leu-Phe-**Gln/Gly**-Pro).

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 Precission 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.

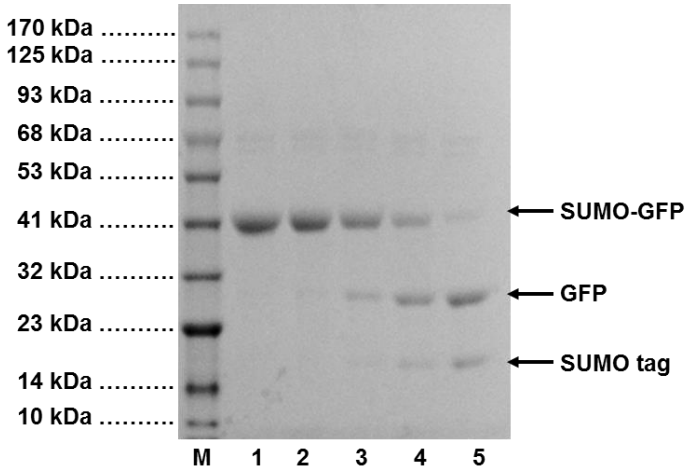

Figure: Analysis of the cleavage activity of SUMO-GFP control protein by EZCut™ HRV14 3C (Precission) Protease Agarose Beads on 4-20% SDS-PAGE after 0 h (**1**), 2 h (**2**), 4 h (**3**), 6 h (**4**) and 24 h (**5**) of reaction at RT (**M**: Protein Marker).

RELATED PRODUCTS:

- Active HRV14 3C Protease Recombinant **(Cat No. 8012-20, -100)**
- HRV 3C Protease Activity Assay Kit (Colorimetric) **(Cat No. K214-100)**
- HRV 3C Protease Inhibitor Screening Kit (Colorimetric) **(Cat No. K215-100)**
- Urokinase Sepharose Beads **(Cat No. 7927-1,-5, -25)**
- Hi-Bind Ni QR Agarose Beads **(Cat No. 6562-1, -10, -100, 500)**
- Thrombin Sepharose Beads **(Cat No. 7925-1,-5, -25)**

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