INSTRUCTIONS

Thermo SCIENTIFIC

Pierce™ HRV 3C Protease

88946 88947

2517.0

Number	Description
88946	**Pierce HRV 3C Protease (1000 units)**
	Kit Contents:
	HRV 3C Protease (2 units/µL), 500µL (1mg/mL)
	GST-Syk Protein, Positive Control, 25µL (1mg/mL)
	10X HRV 3C Reaction Buffer, 10mL
88947	**Pierce HRV 3C Protease (10,000 units)**
	Kit Contents:
	HRV 3C Protease (2 units/µL), 5000µL
	GST-Syk Protein, Positive Control, 25µL
	10X HRV 3C Reaction Buffer, 10mL
	Storage: Upon receipt store at -20°C. Product shipped with dry ice.

Introduction

The Thermo Scientific Pierce HRV 3C Protease is a recombinant cysteine protease from human *rhinovirus* 3C (HRV 3C) expressed in and purified from *Escherichia coli*. Pierce HRV 3C Protease cleaves protein substrates with the recognition sequence Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro between the Gln and Gly residues. The high specificity and dual affinity tags (GST and 6xHis) of the protease make it an ideal choice for the removal of purification and detection tags on recombinant proteins and allows for flexibility in protease removal.

Procedure Summaries

Note: In-solution or on-column cleavage may be used to cleave a protein of interest using HRV 3C protease.

Cleavage in Solution

1. Lyse cells.
2. Purify target protein containing tag using affinity chromatography and elute off the column.
3. Add HRV 3C protease to cleave tag from protein in-solution.
4. Pass cleaved protein over an IMAC or glutathione resin to remove HRV 3C protease contamination.
5. Collect the protein of interest in the flow-through.

On-Column Cleavage

1. Lyse cells.
2. Bind target protein containing tag to an IMAC or glutathione resin.
3. Add HRV 3C protease to the column to cleave the protein tag and release the protein.
4. Collect protein in the flow-through.

Important Product Information

- If the vector does not contain an HRV 3C cleavage site, then two complementary oligonucleotides coding for the HRV 3C recognition sequence must be synthesized. See Table 1 for the possible codons that will code for the HRV 3C recognition sequence, Leu-Glu-Val-Leu-Phe-Gln-Gly-Pro for the cleavage site. This cleavage site sequence must then be inserted into the vector in the proper orientation and reading frame.

Table 1. Possible codons for coding the HRV 3C recognition sequence.

Amino Acid	Leu	Glu	Val	Leu	Phe	Gln	Gly	Pro
DNA Codons	CTT	GAA	GTT	CTT	TTT	CAA	GGT	CCT
	CTC	GAG	GTC	CTC	TTC	CAG	GGC	CCC
	CTA	-	GTA	CTA	-	-	GGA	CCA
	CTG	-	GTG	CTG	-	-	GGG	CCG

Additional Materials Required

- Immobilized glutathione or IMAC resin
- Appropriate purification buffers (binding, wash and elution buffers) depending on purification method used (IMAC or glutathione resin)
- Centrifuge tubes
- Centrifuge

Protocol for HRV 3C Cleavage In-Solution

A. Cleavage Reaction In-Solution

1. Prepare cleavage reaction by adding the previously purified target protein into 10X HRV 3C Reaction Buffer diluted to 1X according to Table 2. A positive control reaction may be prepared side-by-side with the reaction using the GST-Syk Protein.

   Note: HRV 3C protease is active in a variety of different buffers. Optimize the enzyme:substrate ratio when using other purification buffers.

2. Add 1µL (2 units) of HRV 3C protease to the reaction for each up to 200µg of fusion protein.

   Note: Test the proper enzyme:substrate ratio on a small scale before scale-up. Use an enzyme:substrate ratio from 1:200 to 1:5.

3. Incubate the cleavage reaction overnight at 4°C for complete cleavage.

   Note: Completion of the cleavage reaction may be monitored at different time points by removing a portion of the reaction to run on an SDS-PAGE gel.

Table 2. Components for cleavage and positive control reactions.

Component	Fusion Protein	Positive Control
HRV 3C Reaction Buffer (10X)	2.5µL	2.5µL
Target Protein	TBD	-
GST-Syk Protein	-	5µL
HRV 3C Protease	TBD	1µL
Ultrapure water	Up to 25µL	16.5µL
Total	**25µL**	**25µL**

B. Removal of HRV 3C Protease

The resulting cleaved fusion protein may be separated from HRV 3C protease by binding HRV 3C protease (containing both His- and GST tags) to IMAC (nickel, cobalt) or glutathione resins.

1. Equilibrate the appropriate amount of nickel, cobalt or glutathione resin with 2-4 resin-bed volumes of 1X HRV 3C Reaction Buffer in a spin column.
2. Place the spin column into a centrifuge tube.
3. Centrifuge column at 700 × *g* for 2 minutes to remove buffer.
4. Discard the buffer and place the column back into the centrifuge tube.
5. Repeat Steps 2-4 twice for a total of three washes.
6. Plug the bottom of the spin column.
7. Add the cleavage reaction to the resin and cap the top.
8. Incubate for 30-120 minutes at 4°C with end-over-end mixing.
9. Remove bottom plug, place in a new centrifuge tube and centrifuge at 700 × *g*. The cleaved target protein will be found in the resulting flow-through, while the HRV 3C protease will be retained on the column.

Protocol for On-Column HRV 3C Cleavage

A. Purification and Cleavage of Fusion Protein Using IMAC or Glutathione Purification

1. Equilibrate the appropriate amount of nickel, cobalt or glutathione resin with 2-4 resin-bed volumes of 1X HRV 3C Reaction Buffer.

   Note: HRV 3C protease is active in a variety of different buffers. Optimize the enzyme:substrate ratio when using other purification buffers.
2. Place the spin column into a centrifuge tube.
3. Centrifuge column at 700 × *g* for 2 minutes to remove buffer.
4. Discard the buffer and place the column back into the centrifuge tube.
5. Repeat Steps 2-4 twice for a total of three washes.
6. Plug the bottom of the spin column.
7. Mix the target protein with 1X HRV 3C Reaction Buffer such that the total volume equals at least 2 to 3 resin-bed volumes. Other ratios may be used, but need to be empirically determined.
8. Apply diluted target protein to the resin and cap the top.
9. Incubate for 30-120 minutes at 4°C with end-over-end mixing.
10. Remove bottom plug, place in the centrifuge tube and centrifuge at 700 × *g*. Save the flow-through fraction for subsequent purification analysis.
11. Wash the resin by adding 2 resin-bed volumes of wash buffer and collect fraction in a centrifuge tube.
12. Centrifuge at 700 × *g* for 2 minutes. Save the flow-through fraction for subsequent purification analysis.
13. Repeat Steps 11 and 12 twice for a total of three washes.
14. Cap the bottom of the spin column.
15. Resuspend resin in 2 resin-bed volumes of 1X HRV 3C Reaction Buffer.
16. Add the desired amount of HRV 3C protease to the resin slurry.
17. Incubate overnight (≥ 16 hours) at 4°C with end-over-end mixing.
18. Remove bottom cap and place in a centrifuge tube.
19. Centrifuge at 700 × *g*. The cleaved target protein will be found in the resulting flow-through, while the HRV 3C protease will be retained on the column.

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Troubleshooting

Problem	Possible Cause	Solution
No cleavage	Cleavage site was not properly inserted	Sequence plasmid to verify recognition sequence has been inserted properly
		Re-clone recognition sequence into expression vector
	Cleavage site was blocked by protein folding	Optimize folding by changing growth conditions
Incomplete cleavage/minimal cleavage	Suboptimal HRV 3C protease to protein ratio	Increase amount of HRV 3C protease used
	Insufficient incubation time	Increase incubation time
	HRV 3C protease recognition was only partially accessible	Optimize folding by changing growth conditions
		Change cloning strategy to include linkers
	Improper digestion conditions were used (buffer, temperature, time)	Ensure optimal buffers and reaction conditions are used
HRV 3C protease was not eliminated by subtractive purification	Insufficient incubation time	Increase incubation time
	Resin was saturated with HRV 3C protease	Increase amount of resin used
		Pass solution over new resin

Additional Information Available on Our Website

- Tech Tip #4: Batch and spin cup methods for affinity purification of proteins
- Tech Tip #13: Pack beaded affinity resin into columns

Related Thermo Scientific Products

89964-6	**HisPur™ Cobalt Resin**
89967-9	**HisPur Cobalt Spin Columns**
90090-2	**HisPur Cobalt Purification Kits**
88221-2	**HisPur Ni-NTA Resin**
88224-6	**HisPur Ni-NTA Spin Columns**
88227-9	**HisPur Ni-NTA Purification Kits**
16100-2	**Pierce Glutathione Agarose**
16103-5	**Pierce Glutathione Spin Columns**
16106-8	**Pierce Glutathione Purification Kits**
89868-9, 96-8	**Pierce Centrifuge Columns**
32520-01	**Factor Xa**
EO0861*	**WELQut™ Protease**

* Product can be found at *www.thermoscientificbio.com*

General References

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