Recombinant HRV GST-3C Protease Protocol

Cat. # 3CC-N3136

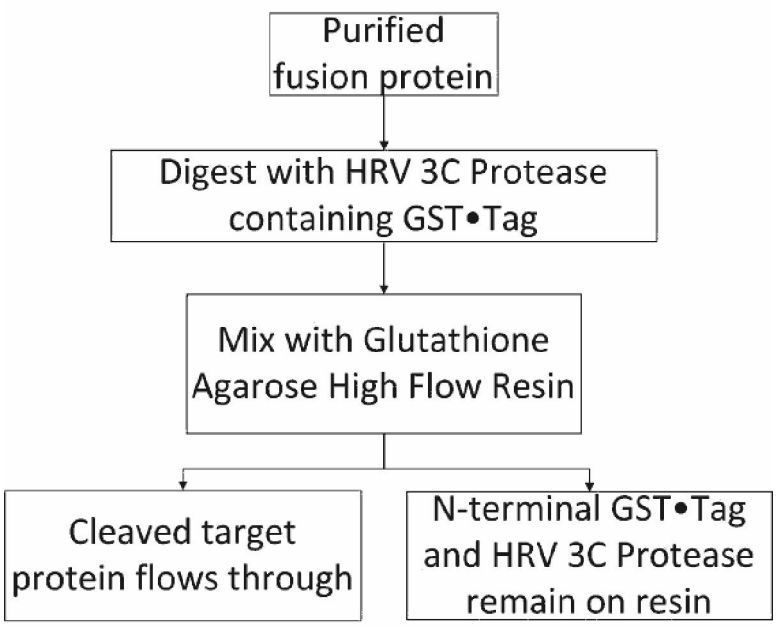
Cleavage Protocol :

1. Make fresh cold cleavage buffer ,recommended typical cleavage buffer is : **50 mM Tris-HCl, pH-7.0 (at 25℃), 150 mM NaCl, 1 mM EDTA, 1 mM dithiothreitol**. Cleavage buffer should be a buffer in which the target protein is soluble. There should be no protease inhibitor in the buffer. The cleavage buffer should be compatible with downstream purification processes, e.g. minimal amount of EDTA or DTT if Ni column will be used to remove the cleaved His-tag. HRV-3C Protease is compatible with 500 mM NaCl and 400 mM imidazole.
2. Dilute the fusion protein pool to **1-2 mg/ml** with cold Cleavage Buffer. This is optional in case the target protein aggregates in the buffer. Keep a small aliquot as Uncut sample(Negative Control) to detect a possible unspecific cleavage either by autolysis or by proteolytic contaminations of the fusion protein.
3. Add GST-HRV 3C protease at a **Protease : Target** protein ratio of **1 :100 (w/w)** (**1,000 unit** GST-HRV 3C Protease to **100 mg target protein**) as initial cleavage condition. The optimal ratio should be determined empirically. A Protease-to-target protein ratio (w/w) of 1:50 to 1:400 should work for most target proteins. There is no need to change buffer or dilute HRV-3C Protease.
4. Incubate the reaction mixture at 4℃ for 16 hours or overnight. If shorter incubation time is required, more amount of HRV-3C protease or higher temperature (RT) should be implemented. When the cleavage conditions are optimized at a small scale, scale up the cleavage proportionally according to specific application requirement.

Removal of HRV GST-3C Protease:

1. Use **Glutathione Agarose High Flow** (ACROBiosystems **Catalog#** GS-0207-01) to remove the HRV GST-3C Protease.
2. If desired, determine and compare the extent of cleavage of the samples by SDS-PAGE analysis.

Experimental Outline:



Factors that Influence HRV GST-3C Activity:

Depending on the buffers used and their chemical components, HRV GST-3C Protease cleavage efficiency may be affected. The following table shows the relative activity of HRV GST-3C Protease under various conditions.

Factor Type	Component	Condition	Relative 3C Protease activity
Salt	NaCl	0.8 M	150
		0.2 M	110
		2.5-3.0 M	200
	$ZnCl_2$	0.2 M	0
Protease inhibitor	EDTA	50 mM	100
	EGTA	50 mM	100
	Egg White Cystatin	8.0 μM	100
	E-64 Protease Inhibitor	100 μM	100
	Iodoacetamide	1.0±0.1 mM	50
	Pepstatin	20 μM	100
	Aprotinin	15 μM	100
	Benzamidine	50 mM	100
	Leupeptin	0.75±0.05 mM	50
	PMSF	8.0 mM	50
	TLCK	1.0 mM	50
Denaturant	Urea	3 M	0
		2 M	0
		1 M	40
	Guanadine	3 M	0
		2 M	0
		1 M	0
Reductant	DTT	1 mM	100
Detergent	Triton X-100	0.10%	>100
		1%	100
	Tween-20	0.10%	>100
		1%	100
	Nonidet P-40	0.10%	>100
		1%	100
Anion(Na salt)	Cl^-	0.2 M	110
		0.4 M	130
		0.8 M	150
	I^-	0.2 M	81
		0.4 M	63
		0.8 M	54
	SO_4^{2-}	0.2 M	252
		0.4 M	680
		0.8 M	1570
Co-solvent	Acetonitrile	10%	48
	DMSO	10%	74
	Isopropanol	10%	74
	Methanol	10%	91
	Glycerol	10%	114
	Etylene glycol	10%	95
	PEG-3400	10%	90
	Sorbitol	10%	120
	Sucrose	10%	112