

MRC PPU Reagents and Services

Standard Operating Procedure

Preparation of FLIP [1 – 480]

Enzyme description:- FLIP [1 – 480]

Clone number:- DU 23321

Source:- Recombinant

Expression system:- *E.coli*,

Tag:- N-terminal MBP

Purification method:- Amylose resin

Calculated molecular mass:-

Monoisotopic 103, 062.00 daltons

Average Mass 103, 127.26 daltons

[cysteines reduced, methionines have not been oxidised]

Theoretical pI:- 6.21

Purity:- >80 %

Enzyme storage buffer:-

50 mM Tris-HCl pH 7.5, 150 mM NaCl, 270 mM sucrose,
0.1 % 2-mercaptoethanol, 1 mM benzamidine, 0.2 mM PMSF

Storage temperature:- -70 °C

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Clone Data Sheet

FLIP [1 - 480]

Protein FLIP [1 - 480]

Clone number DU 23321

Species Human

Accession number O15519 - 1

Tags N-terminal MBP

**Bacterially
expressed FLIP
protein**

MKIKTGARILALSALTTMMFSASALAKIEEGKLVWINGDKGYNGLAE
VGKKFEKDTGIKVTVEHPDKLEEKFPQVAATGDGPDIFWAHDRFGGY
AQSGLLAEITPDKAFQDKLYPFTWDAVRYNGKLIAYPIAVEALSLIYN
KDLLPNPPKTWEEIPALDKELKAKGKSALMFNLQEPYFTWPLIAADGG
YAFKYENKDYDIKDVGVNAGAKAGLTFVLVDLIKHKHMNADTDYSIAE
AAFNKGETAMTINGPWAWSNIDTSKVNYGVTVLPTFKGQPSKPFVGVL
SAGINAASPNKELAKEFLENYLLTDEGLEAVNKDKPLGAVALKSYEEE
LAKDPRIAATMENAQKGEIMPNI PQMSAFWYAVRTAVINAASGRQTV
EALKDAQTNSSNNNNNNNNNNLGI EGRI SELALEVLFQGPWGSPEFP
GVDSAAAMSAEVIHQVEEALDTDEKEMLLFLCRDVAIDVVPNVRDLL
DILRERKLSVGD LAELLYRVRREFDLLKRI LKMDRKAVETHLLRNPHL
VSDYRVLMAEIGEDLDKSDVSSLI FLMKDYMGRGKISKEKSFLDLVVE
LEKLNLVAPDQLDLLEKCLKNIHRIDLKTKIQKYKQSVQAGTSYRNV
LQAAIQKSLKDP SNNFRLHNGRSKEQRLKEQLGAQQEPVKKSIQES
EALPQSIPEERYKMKSKPLGICLI IDCIGNETELLRDTFTSLGYEVQKF
LHLSMHGISQILGQFACMPEHRDYDSFVCVLSRGGSSQSVYGVQDTHS
GLPLHHIRRMFMGDSCPYLAGPKMFFIQNYVVSEGQLEDSSLLEVDG
PAMKNVEFKAQKRGLCTVHREADFFWSLCTADMSLLEQSHSSPSLYLQ
CLSQKLRQERKRPLLDLHIELNGYMYDWNRSRVSACEKYYVWLQHTLRK
KLILSYT*

Native sequence Amino acids M1 – T480 (end) of human FLIP.
Residue M440 of the fusion protein is equivalent to M1 of the native
enzyme. The MBP tag is located at residues 1 - 367.

Protease cleavage Factor X (IEGR) residues 409 – 412

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