

Mechanism of the Antigen-Independent Cytokinergic SPE-7 IgE Activation of Human Mast Cells *in Vitro*

Heather J Bax, Holly Bowen, Tihomir S Dodev, Brian J Sutton, Hannah J Gould

Supplementary Information:

Supplementary Table – Primers

Supplementary Figure 1 – SPE-7 IgE Fab Amino Acid Sequence

Supplementary Figure 2 – SPE-7 IgE Fab Cloning, Expression and Purification

Supplementary Table - Primers

Where primers were used to add a His-Tag or homologous ends, these regions are underlined.

Primer	Sequence
<i>SPE-7 IgE Heavy and light chain amplification from NS1 hybridoma cells</i>	
SPE-7_L_F	CAGGCTGTTGTGACTCAGGAATC
SPE-7_L_R	CTAGGAACAGTCAGCACGGGA
SPE-7_H_F	GAGGTGCAGCTGCAGCAG
SPE-7_H_R	CTAGGAGGGACGGAGGGAG
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<i>SPE-7 IgE Fab heavy chain amplification and His-Tag addition for PIPE cloning</i>	
Fab_H_R1_F	GCGAATTCGCCACCATGGACT
Fab_H_R1_R	<u>CTAGTGATGGTGATGGTGATGGACAGGTCGAACTAGGAT</u>
Fab_H_R2_F	<u>CGCTAATTCAAAGCAAATGGACTGGACCTGGAGGATCCTC</u>
Fab_H_R2_R	<u>ATGTCTGGCCAGCTAGCTAGTGATGGTGATGGTGATGGAC</u>
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<i>SPE-7 IgE Light chain amplification for PIPE cloning</i>	
BspEI_L_F	<u>GCTAATTCAAAGCAAATATGTTGCCATCACA</u> ACTCATTGGG
AvrII_L_R	GGAAACCTGCTCCTAGCTAGACTAGGAACAGTCAGCACGG

SPE-7 IgE Fab Heavy Chain

←
EVQLQQPGAE LVKPGASVKL SCKASGYTFT SYWMHWVKQR PGRGLEWIGR

SPE-7 VH _____
IDPNGGGTKY NEKFKSKATL TVDKPSSTAY MQLSSLTSED SAVYYCARMW

YYGTYFDYW GQGTTLVSS ASIRNPQLYP LKPKGTASM TLGCLVKDYF

Murine Cε1 _____
PGPVTVTWYS DSLNMSTVNF PALGSELKVT TSQVTSWGKS AKNFTCHVTH

PPSFNESRTI LVRPV
→

SPE-7 IgE Fab Light Chain

←
QAVVTQESAL TTSPGETVTL TCRSSTGAVT TSNYANWVQE KPDHLFTGLI

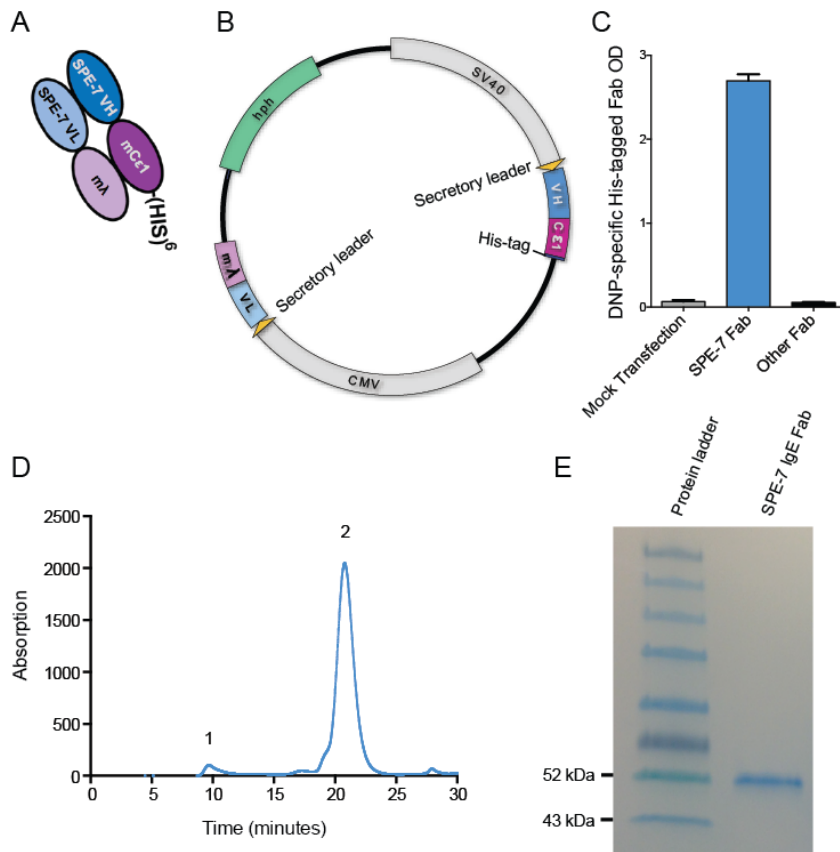
SPE-7 VL _____
GGTNNRAPGV PARFSGSLIG DKAALTITGA QTEDEAIYFC ALWYSNHLVF

GGGTKLTVLG QPKSSPSVTL FPPSSEELET NKATLVCTIT DFYPGVVTVD

Murine Cλ _____
WKVDGTPVTQ GMETTQPSKQ SNNKYMSSY LTLTAGAWER HNSYSCQVTH

EGHTVEKSLS RADCS
→

Supplementary Figure 1– SPE-7 IgE Fab Amino Acid Sequence
SPE-7 IgE Fab heavy and light chain amino acid sequences (GenBank Accession Nos. KJ734989 and KJ734990), derived from NS1 hybridoma cells, and cloned and expressed as SPE-7 IgE Fab protein.



Supplementary Figure 2 – SPE-7 IgE Fab Cloning, Expression and Purification

Schematic of the SPE-7 IgE Fab construct (A) and pVITRO1 vector containing IgE Fab heavy chain (VH and Cε1) and light chain (VL and CL) used to transfect HEK293F cells for mammalian expression (B). Anti-his-tag ELISA to measure expression of DNP-specific SPE-7 IgE Fab by HEK293F cells (C). Size-exclusion chromatography purification profile (D). Monomeric IgE Fab was eluted at around 20 minutes (2) and these fractions collected to remove aggregated protein (1). SDS-PAGE analysis of SPE-7 IgE Fab protein under non-reducing conditions (E).