Table 1
The recombinant FV variants; primers and restriction enzymes used.

Mutant	Template	Primer	Enzymes
R316A/H318A	WT-FV	5'-GTG AGC AG**G** **C**GC GG**G** **C**CA TGA AGA GG-3'	-
R316A/H318A/R400A	R316A/H318A	5'- TCA GAG CCC AGG TC**G** **C**AG ACA CAC TC-3'	Xcm/Bsu 36I (0.5 kb)
K320A/R321A/E323A	WT-FV	5'-GGC ACA TG**G** **C**G**G** **C**GT GGG **C**AT TCA TTG C-3'	Xcm/Bsu 36I (0.5 kb)
E329A/E330A	WT-FV	5'-TCA TTG CTG CAG **C**GG **C**AG TCA TTT GGG-3'	Xcm/Bsu 36I (0.5 kb)
E329A/E330A/K379A	E329A/E330A	5'-GAT GAG TCC TTC ACC AAA **GC**T ACA GTG AAT CCC-3'	Xcm/Bsu 36I (0.5 kb)
K386A	WT-FV	5'-GTG AAT CCC AAT ATG **GC**A GAA GAT GGG ATT TTG GG-3'	-
K386A/R652A/K655A	K386A	5'-GCT GAA ATT C**GC** GGA TGT T**GC** ATG TAT CCC AG -3'	Xcm/BspEI (2.5 kb)
E461A/E467A	WT-FV	5'-AAC ATC TTA G**C**G TTT GAT GAA CCC ACA G**C**A AAT GAT GCC C-3'	-
E461A/E467A/K499A	E461A/E467A	5'-CTT CTA ATC TGT **GC**G AGC AGA TCC CTG G-3'	Bsu 36I/BspEI (2 kb)
R501A	WT-FV	5'-CTG TAA GAG C**GC** ATC CCT GGA CAG G-3'	Bln1/Bsu 36I (2 kb)
R510A	WT-FV	5'-CGA GGA ATA CAG **GC**G GCA GCA GAC ATC G-3'	Bln1/Bsu 36I (2 kb)
A511D	WT-FV	5'-GGA ATA CAG AGG G**AC** GCA GAC ATC GAA CAG C-3'	Bln1/Bsu 36I (2 kb)
R501A/R510A/A511D	R501A	5'-AGG AAT ACA G**GC** GG**A** **C**GC AGA CAT CG-3'	Bsu 36I/BspEI (2 kb)
D513A	WT-FV	5'-AGG GCA GCA G**C**C ATC GAA CAG CAG GC-3'	-
R501A/R510A/A511D/D513A	R501A/R510A/A511D	5'-CAG GCG GAC GCA G**C**C ATC GAA CAG CAG GC-3'	Bsu 36I/BspEI (2 kb)
D513A/D577A/D578A	D513A	5'-GAT TCT GCT TTG **C**TG **C**CA CTG TCC AGT GG-3'	Bsu 36I/BspEI (2 kb)
D577A/D578A	WT-FV	5'-GAT TCT GCT TTG **C**TG **C**CA CTG TCC AGT GG-3'	Bsu 36I/BspEI (2 kb)
R1551A	WT-FV	5'-CGC AGC AAC AAT GGA AAC **GC**A AGA AAT TAT TAC ATT GCT GC-3'	-
R1551A/H1683E	R1551A	5'-TAT ACC TAC GTA TGG **GA**G GCC ACT GAG CG-3'	-
R1551A/ E1650A/H1683E	R1551A/H1683E	5'-CCC ATG GAC TTT CCT ATG **C**AA AAT CAT CAG AGG G-3'	Kpn 1 (9kb)
E1650A	WT-FV	5'-CCC ATG GAC TTT CCT ATG **C**AA AAT CAT CAG AGG G-3'	-
E1650A/W1665A	E1650A	5'-GAT GAC TCT CCT GAA **GC**G TTT AAG GAA GAT AAT GCT G-3'	Kpn 1 (9kb)

Sense primers used for mutagenesis and restriction enzymes employed for isolating the fragments containing the mutations, which were then used to replace the corresponding fragment in WT FV cDNA. Changes in nucleotide sequences are in bold, antisense counterpart primers are not shown. The size of each cleaved fragment that was isolated and ligated into the WT FV cDNA lacking the corresponding fragment is shown in brackets. The investigated variants are in bold and underlined.