# BLI-based Assessment of RBD-hACE2 Binding Interactions

# Sample Preparation Data

| Accession | Experimental ID | Construct ID | Accession | Experimental ID | Construct ID | Accession | Experimental ID | Construct ID |
|---|---|---|---|---|---|---|---|---|
| SARS_RBD | mut1 | RBD-mut1-FC(IgG1) | FJ647226 | mut16 | RBD-mut16-FC(IgG1) | OK017820 | mut25 | RBD-mut25-FC(IgG1) |
| KU182965 | mut2 | RBD-mut2-FC(IgG1) | FJ647220 | mut17 | RBD-mut17-FC(IgG1) | OK017825 | mut26 | RBD-mut26-FC(IgG1) |
| EF065508 | mut3 | RBD-mut3-FC(IgG1) | FJ647223 | mut18 | RBD-mut18-FC(IgG1) | OK017826 | mut27 | RBD-mut27-FC(IgG1) |
| EF065505 | mut4 | RBD-mut4-FC(IgG1) | MW532698 | mut19 | RBD-mut19-FC(IgG1) | OK017844 | mut28 | RBD-mut28-FC(IgG1) |
| EF065506 | mut5 | RBD-mut5-FC(IgG1) | MT121216 | mut20 | RBD-mut20-FC(IgG1) | OK017852 | mut29 | RBD-mut29-FC(IgG1) |
| EF065507 | mut6 | RBD-mut6-FC(IgG1) | OK017848 | mut21 | RBD-mut21-FC(IgG1) | OK017856 | mut30 | RBD-mut30-FC(IgG1) |
| MN611519 | mut7 | RBD-mut7-FC(IgG1) | OK017850 | mut21 | RBD-mut21-FC(IgG1) | OK017853 | mut30 | RBD-mut30-FC(IgG1) |
| MG916903 | mut8 | RBD-mut8-FC(IgG1) | OK017851 | mut21 | RBD-mut21-FC(IgG1) | OK017855 | mut30 | RBD-mut30-FC(IgG1) |
| JQ989266 | mut9 | RBD-mut9-FC(IgG1) | OK017847 | mut21 | RBD-mut21-FC(IgG1) | OK017854 | mut31 | RBD-mut31-FC(IgG1) |
| JQ989267 | mut10 | RBD-mut10-FC(IgG1) | OK017849 | mut22 | RBD-mut22-FC(IgG1) | OK017857 | mut32 | RBD-mut32-FC(IgG1) |
| JQ989268 | mut11 | RBD-mut11-FC(IgG1) | OK017814 | mut23 | RBD-mut23-FC(IgG1) | OK017797 | mut33 | RBD-mut33-FC(IgG1) |
| JQ989269 | mut12 | RBD-mut12-FC(IgG1) | OK017816 | mut23 | RBD-mut23-FC(IgG1) | KT444582 | mut34 | RBD-mut34-FC(IgG1) |
| KF294371 | mut13 | RBD-mut13-FC(IgG1) | OK017817 | mut23 | RBD-mut23-FC(IgG1) | KY417150 | mut35 | RBD-mut35-FC(IgG1) |
| AC_000192 | mut14 | RBD-mut14-FC(IgG1) | OK017821 | mut23 | RBD-mut23-FC(IgG1) | KF367457 | mut36 | RBD-mut36-FC(IgG1) |
| FJ647219 | mut15 | RBD-mut15-FC(IgG1) | OK017815 | mut24 | RBD-mut24-FC(IgG1) | KY417151 | mut37 | RBD-mut37-FC(IgG1) |

SARS_RBD and SARS2_RBD denote the reference RBD sequences of SARS-CoV and SARS-CoV-2 used for experimental comparison.

# Sample Preparation Data

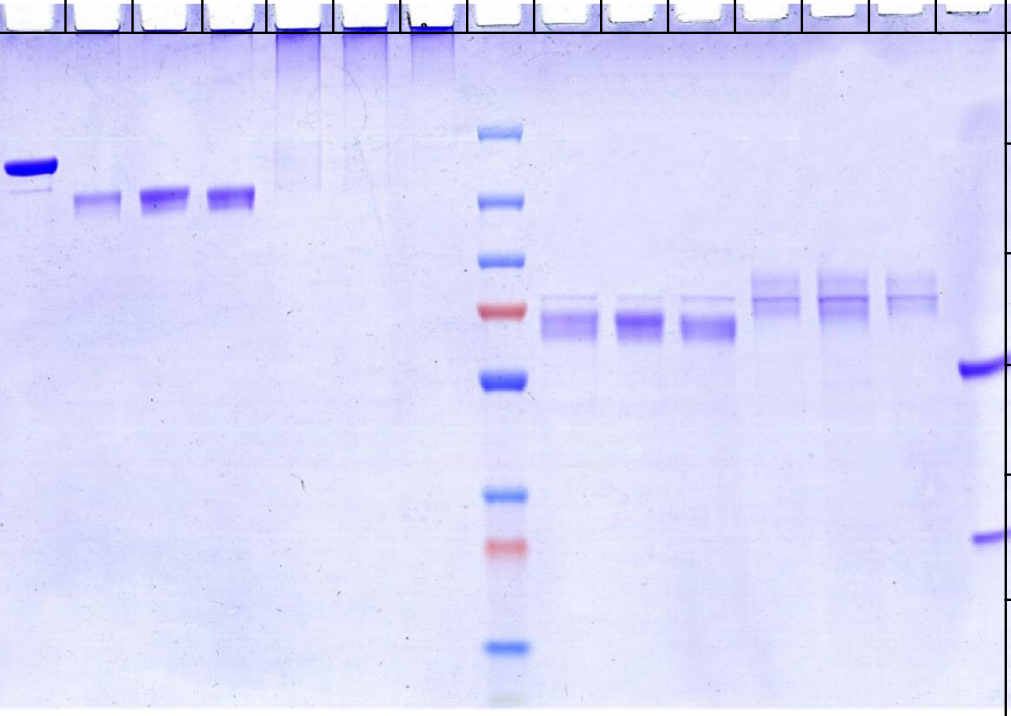| Accession | Experimental ID | Construct ID | Accession | Experimental ID | Construct ID | Accession | Experimental ID | Construct ID |
|---|---|---|---|---|---|---|---|---|
| MW681002 | mut38 | RBD-mut38-FC(IgG1) | GQ153541 | mut51 | RBD-mut51-FC(IgG1) | MZ937000 | mut64 | RBD-mut64-FC(IgG1) |
| MG772933 | mut39 | RBD-mut39-FC(IgG1) | GQ153544 | mut52 | RBD-mut52-FC(IgG1) | KC881005 | mut65 | RBD-mut65-FC(IgG1) |
| AY278489 | mut40 | RBD-mut40-FC(IgG1) | GQ153545 | mut52 | RBD-mut52-FC(IgG1) | EPI_ISL_410538 | mut66 | RBD-mut66-FC(IgG1) |
| AY572034 | mut41 | RBD-mut41-FC(IgG1) | GQ153546 | mut52 | RBD-mut52-FC(IgG1) | EPI_ISL_410539 | mut67 | RBD-mut67-FC(IgG1) |
| AY572035 | mut42 | RBD-mut42-FC(IgG1) | KP886808 | mut53 | RBD-mut53-FC(IgG1) | EPI_ISL_410540 | mut68 | RBD-mut68-FC(IgG1) |
| AY686863 | mut43 | RBD-mut43-FC(IgG1) | KP886809 | mut54 | RBD-mut54-FC(IgG1) | EPI_ISL_410541 | mut69 | RBD-mut69-FC(IgG1) |
| AY686864 | mut44 | RBD-mut44-FC(IgG1) | KF294457 | mut55 | RBD-mut55-FC(IgG1) | EPI_ISL_410721 | mut70 | RBD-mut70-FC(IgG1) |
| AY572038 | mut44 | RBD-mut44-FC(IgG1) | EU371561 | mut56 | RBD-mut56-FC(IgG1) | EPI_ISL_471467 | mut71 | RBD-mut71-FC(IgG1) |
| DQ071615 | mut45 | RBD-mut45-FC(IgG1) | EU371562 | mut57 | RBD-mut57-FC(IgG1) | EPI_ISL_471470 | mut72 | RBD-mut72-FC(IgG1) |
| MG772934 | mut46 | RBD-mut46-FC(IgG1) | EU371563 | mut58 | RBD-mut58-FC(IgG1) | C_AA085190 | mut73 | RBD-mut73-FC(IgG1) |
| DQ022305 | mut47 | RBD-mut47-FC(IgG1) | KJ473815 | mut59 | RBD-mut59-FC(IgG1) | C_AA085189 | mut73 | RBD-mut73-FC(IgG1) |
| DQ084200 | mut47 | RBD-mut47-FC(IgG1) | MZ937001 | mut60 | RBD-mut60-FC(IgG1) | C_AA085191 | mut74 | RBD-mut74-FC(IgG1) |
| DQ084199 | mut48 | RBD-mut48-FC(IgG1) | MZ937002 | mut61 | RBD-mut61-FC(IgG1) | C_AA085192 | mut75 | RBD-mut75-FC(IgG1) |
| GQ153539 | mut49 | RBD-mut49-FC(IgG1) | MZ937003 | mut62 | RBD-mut62-FC(IgG1) | C_AA085194 | mut76 | RBD-mut76-FC(IgG1) |
| GQ153540 | mut50 | RBD-mut50-FC(IgG1) | MZ937004 | mut63 | RBD-mut63-FC(IgG1) | SARS2_RBD | mut77 | RBD-mut77-FC(IgG1) |

**SARS_RBD and SARS2_RBD denote the reference RBD sequences of SARS-CoV and SARS-CoV-2 used for experimental comparison.**

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 4 | JKY023 | RBD-mut1-FC(IgG1) | P308333 | Receptor binding domain | 102.18 | >85.0 |
| 5 | JKY023 | RBD-mut2-FC(IgG1) | P308334 | Receptor binding domain | 104.84 | >85.0 |
| 6 | JKY023 | RBD-mut3-FC(IgG1) | P308335 | Receptor binding domain | 104.36 | >85.0 |
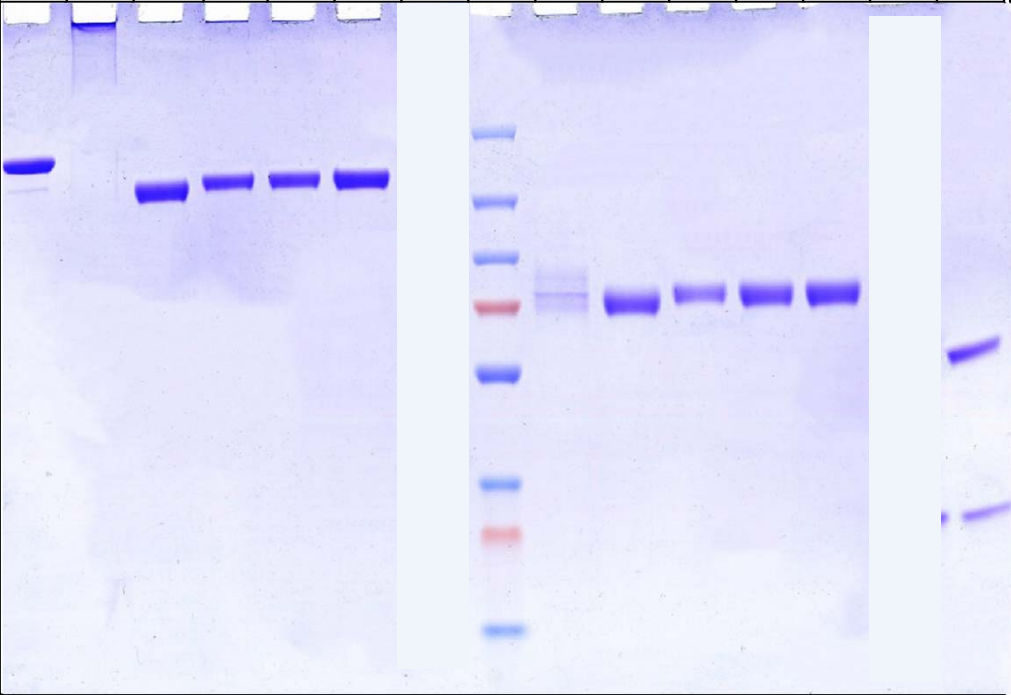
# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1 | JKY023 | RBD-mut4-FC(IgG1) | P308336 | Receptor binding domain | 104.50 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut5-FC(IgG1) | P308337 | Receptor binding domain | 104.48 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut6-FC(IgG1) | P308338 | Receptor binding domain | 104.44 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut9-FC(IgG1) | P308341 | Receptor binding domain | 113.28 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut10-FC(IgG1) | P308342 | Receptor binding domain | 113.28 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut11-FC(IgG1) | P308343 | Receptor binding domain | 113.34 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)

| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | | | | | | | |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut7-FC(IgG1) | P308339 | Receptor binding domain | 104.68 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut8-FC(IgG1) | P308340 | Receptor binding domain | 113.46 | >85.0 |
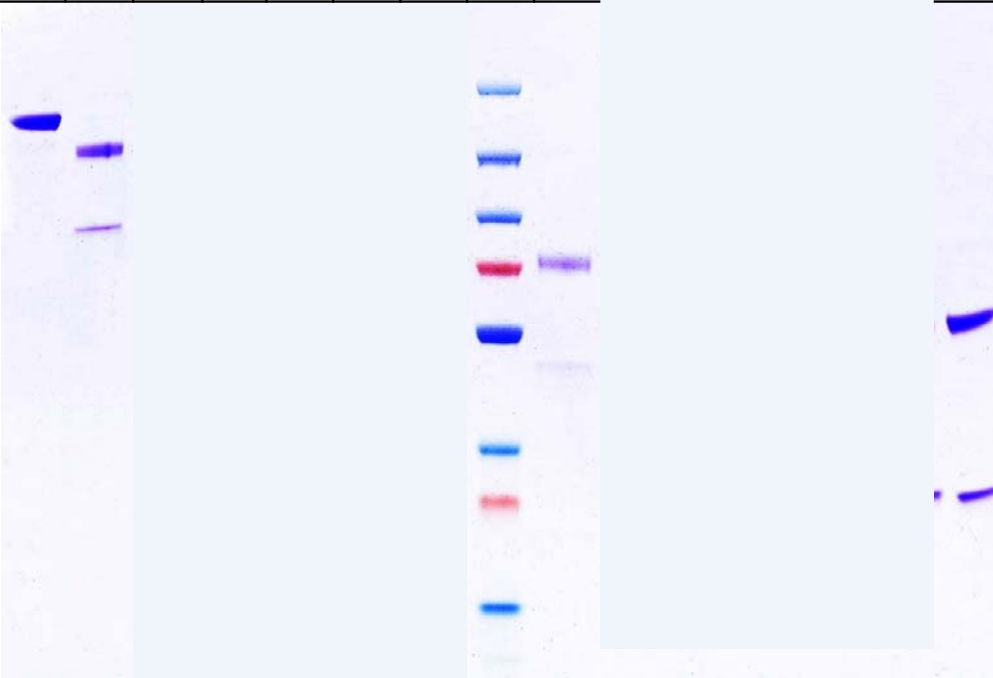
kDa

185
115
80
65
50
30
25
15
10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 1 | JKY023 | RBD-mut12-FC(IgG1) | P308344 | Receptor binding domain | 113.28 | >85.0 |
| 2 | JKY023 | RBD-mut13-FC(IgG1) | P308345 | Receptor binding domain | 120.62 | >85.0 |
| 3 | JKY023 | RBD-mut14-FC(IgG1) | P308346 | Receptor binding domain | 123.68 | >85.0 |
| 4 | JKY023 | RBD-mut15-FC(IgG1) | P308347 | Receptor binding domain | 123.68 | >85.0 |
| 5 | JKY023 | RBD-mut17-FC(IgG1) | P308349 | Receptor binding domain | 123.68 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 6 | JKY023 | RBD-mut16-FC(IgG1) | Receptor binding domain | Antigen | 123.62 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1 | JKY023 | RBD-mut18-FC(IgG1) | P308350 | Receptor binding domain | 120.50 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 4 | JKY023 | RBD-mut19-FC(IgG1) | P308351 | Receptor binding domain | 102.08 | >95.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | N/A | N/A | N/A | 5 | 6 | | 1 | N/A | N/A | N/A | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | | | | | | | |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut20-FC(IgG1) | P308352 | Receptor binding domain | 102.32 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | | | | | | | |
| | | | | | | | | | | | | | | | | 1 | JKY023 | RBD-mut22-FC(IgG1) | P308354 | Receptor binding domain | 102.08 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut23-FC(IgG1) | P308355 | Receptor binding domain | 98.48 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut24-FC(IgG1) | P308356 | Receptor binding domain | 98.48 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut25-FC(IgG1) | P308357 | Receptor binding domain | 98.48 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut26-FC(IgG1) | P308358 | Receptor binding domain | 98.52 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut27-FC(IgG1) | P308359 | Receptor binding domain | 98.52 | >85.0 |

kDa
185
115
80
65
50
30
25
15
10

# SDS-PAGE (Gel concentration:4~20%)
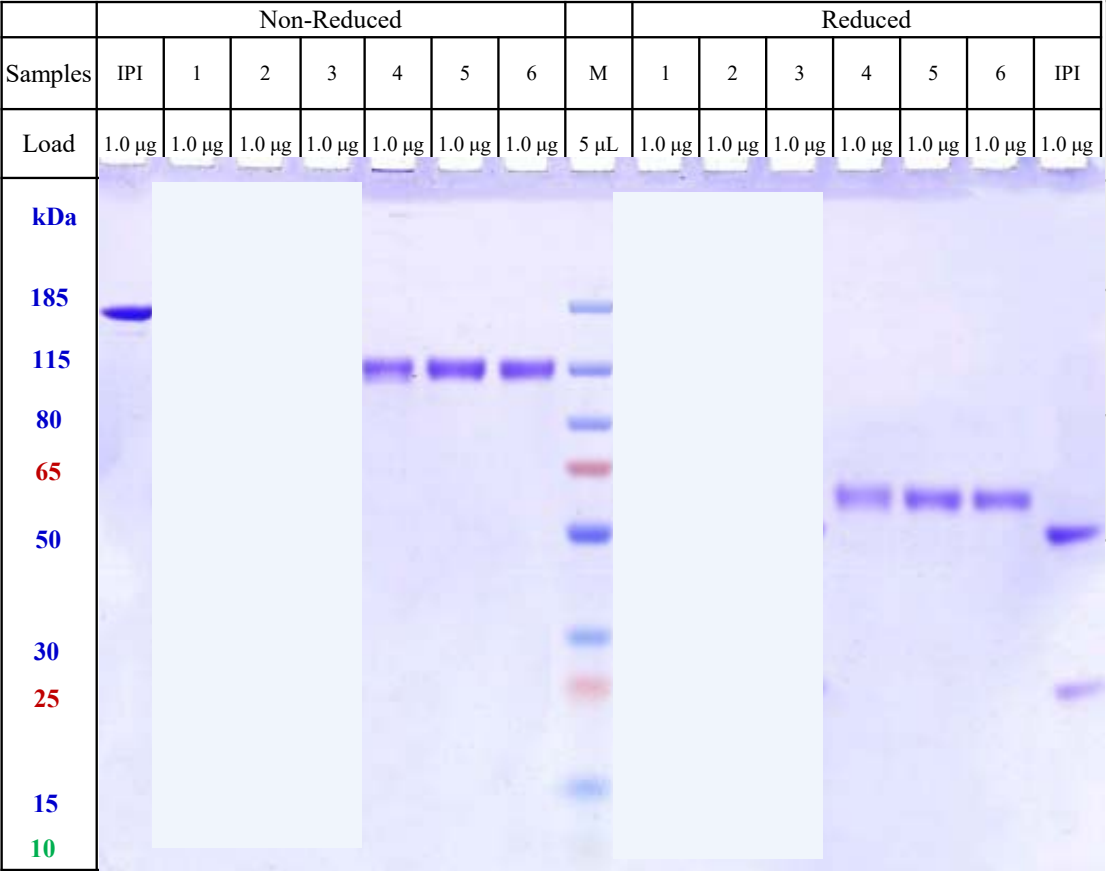


| Samples | | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Non-Reduced | | | | | | | | Reduced | | | | | | |
| Load | | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 4 | JKY023 | RBD-mut28-FC(IgG1) | P308360 | Receptor binding domain | 98.40 | >85.0 |
| 5 | JKY023 | RBD-mut31-FC(IgG1) | P308363 | Receptor binding domain | 101.58 | >85.0 |
| 6 | JKY023 | RBD-mut32-FC(IgG1) | P308364 | Receptor binding domain | 101.58 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | | | | | | | |
| | | | | | | | | | | | | | | | | 1 | JKY023 | RBD-mut36-FC(IgG1) | P308368 | Receptor binding domain | 102.16 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut35-FC(IgG1) | P308367 | Receptor binding domain | 102.16 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut34-FC(IgG1) | P308366 | Receptor binding domain | 102.16 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut33-FC(IgG1) | P308365 | Receptor binding domain | 98.22 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut37-FC(IgG1) | P308369 | Receptor binding domain | 102.08 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut38-FC(IgG1) | P308370 | Receptor binding domain | 98.58 | >85.0 |

kDa markers: 185, 115, 80, 65, 50, 30, 25, 15, 10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1 | JKY023 | RBD-mut39-FC(IgG1) | P308371 | Receptor binding domain | 98.50 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut40-FC(IgG1) | P308372 | Receptor binding domain | 102.46 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut41-FC(IgG1) | P308373 | Receptor binding domain | 102.00 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut42-FC(IgG1) | P308374 | Receptor binding domain | 102.00 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | ACE2-His | P308410 | Receptor | 69.91 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 3 | JKY023 | RBD-mut45-FC(IgG1) | P308377 | Receptor binding domain | 98.54 | >85.0 |
| 4 | JKY023 | RBD-mut46-FC(IgG1) | P308378 | Receptor binding domain | 98.58 | >85.0 |
| 5 | JKY023 | RBD-mut47-FC(IgG1) | P308379 | Receptor binding domain | 98.54 | >85.0 |
| 6 | JKY023 | RBD-mut48-FC(IgG1) | P308380 | Receptor binding domain | 98.54 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 1 | JKY023 | RBD-mut66-FC(IgG1) | P308398 | Receptor binding domain | 102.08 | >85.0 |
| 2 | JKY023 | RBD-mut68-FC(IgG1) | P308400 | Receptor binding domain | 102.08 | >85.0 |
| 3 | JKY023 | RBD-mut49-FC(IgG1) | P308381 | Receptor binding domain | 98.54 | >85.0 |
| 4 | JKY023 | RBD-mut50-FC(IgG1) | P308382 | Receptor binding domain | 98.54 | >85.0 |
| 5 | JKY023 | RBD-mut51-FC(IgG1) | P308383 | Receptor binding domain | 98.54 | >85.0 |
| 6 | JKY023 | RBD-mut52-FC(IgG1) | P308384 | Receptor binding domain | 98.54 | >85.0 |

Gel lane labels:

| | Non-Reduced | | | | | | | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | IPI | 1 | 2 | 3 | N/A | 5 | 6 | M | 1 | 2 | 3 | N/A | 5 | 6 | IPI |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg |

kDa markers: 185, 115, 80, 65, 50, 30, 25, 15, 10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Samples | IPI | 1 | 2 | 3 | 4 | 5 | N/A | M | 1 | 2 | 3 | 4 | 5 | N/A | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1 | JKY023 | RBD-mut53-FC(IgG1) | P308385 | Receptor binding domain | 98.28 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut54-FC(IgG1) | P308386 | Receptor binding domain | 98.28 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut55-FC(IgG1) | P308387 | Receptor binding domain | 98.60 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut56-FC(IgG1) | P308388 | Receptor binding domain | 102.18 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut57-FC(IgG1) | P308389 | Receptor binding domain | 102.18 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut58-FC(IgG1) | P308390 | Receptor binding domain | 102.18 | >85.0 |

kDa
185
115
80
65
50
30
25
15
10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | N/A | N/A | N/A | 5 | 6 | | 1 | N/A | N/A | N/A | 5 | 6 | IPI |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 6 | JKY023 | RBD-mut59-FC(IgG1) | P308391 | Receptor binding domain | 98.48 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | | | | | | | |
| | | | | | | | | | | | | | | | | 1 | JKY023 | RBD-mut60-FC(IgG1) | P308392 | Receptor binding domain | 102.24 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut61-FC(IgG1) | P308393 | Receptor binding domain | 98.50 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut62-FC(IgG1) | P308394 | Receptor binding domain | 102.24 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut63-FC(IgG1) | P308395 | Receptor binding domain | 98.50 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut64-FC(IgG1) | P308396 | Receptor binding domain | 102.26 | >85.0 |
| | | | | | | | | | | | | | | | | 6 | JKY023 | RBD-mut65-FC(IgG1) | P308397 | Receptor binding domain | 101.62 | >85.0 |

kDa

185

115

80

65

50

30

25

15

10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | N/A | N/A | 6 | | 1 | 2 | 3 | N/A | N/A | 6 | IPI | | | | | | | |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1 | JKY023 | RBD-mut67-FC(IgG1) | P308399 | Receptor binding domain | 102.08 | >85.0 |
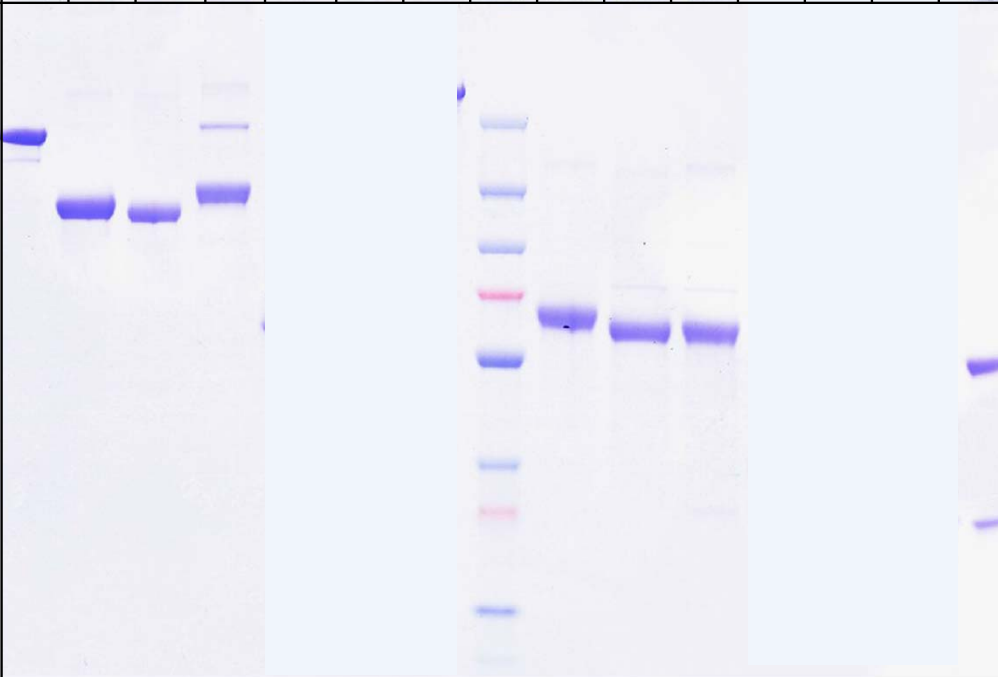
kDa

185

115

80

65

50

30

25

15

10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | N/A | 6 | | 1 | 2 | 3 | 4 | N/A | 6 | IPI |
| Load | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 5 µL | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg | 1.0 µg |

kDa: 185, 115, 80, 65, 50, 30, 25, 15, 10

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 1 | JKY023 | RBD-mut29-FC(IgG1) | P308361 | Receptor binding domain | 101.60 | >85.0 |
| 2 | JKY023 | RBD-mut43-FC(IgG1) | P308375 | Receptor binding domain | 102.00 | >85.0 |
| 3 | JKY023 | RBD-mut44-FC(IgG1) | P308376 | Receptor binding domain | 102.02 | >85.0 |
| 4 | JKY023 | RBD-mut21-FC(IgG1) | P308353 | Receptor binding domain | 102.16 | 57.8 |
| 6 | JKY023 | RBD-mut69-FC(IgG1) | P308401 | Receptor binding domain | 102.20 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | M | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1 | JKY023 | RBD-mut70-FC(IgG1) | P308402 | Receptor binding domain | 102.30 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut71-FC(IgG1) | P308403 | Receptor binding domain | 102.26 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut72-FC(IgG1) | P308404 | Receptor binding domain | 102.30 | >85.0 |
| | | | | | | | | | | | | | | | | 4 | JKY023 | RBD-mut73-FC(IgG1) | P308405 | Receptor binding domain | 102.28 | >85.0 |
| | | | | | | | | | | | | | | | | 5 | JKY023 | RBD-mut74-FC(IgG1) | P308406 | Receptor binding domain | 102.20 | >85.0 |

kDa: 185, 115, 80, 65, 50, 30, 25, 15, 10

# SDS-PAGE (Gel concentration:4~20%)



| Samples | Non-Reduced | | | | | | | M | Reduced | | | | | | | No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI | | | | | | | |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | | | | | | | |
| | | | | | | | | | | | | | | | | 1 | JKY023 | RBD-mut75-FC(IgG1) | P308407 | Receptor binding domain | 102.20 | >85.0 |
| | | | | | | | | | | | | | | | | 2 | JKY023 | RBD-mut76-FC(IgG1) | P308408 | Receptor binding domain | 102.24 | >85.0 |
| | | | | | | | | | | | | | | | | 3 | JKY023 | RBD-mut77-FC(IgG1) | P308409 | Receptor binding domain | 102.34 | >85.0 |

# SDS-PAGE (Gel concentration:4~20%)



| Samples |  | Non-Reduced | | | | | | M | Reduced | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | IPI | 1 | 2 | 3 | 4 | 5 | 6 | | 1 | 2 | 3 | 4 | 5 | 6 | IPI |
| Load | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 5 μL | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg | 1.0 μg |

| No | Project No. | Sample | Protein No. | Protein Type | MW (kDa) | Purity (%) |
|---|---|---|---|---|---|---|
| 5 | JKY023 | RBD- mut30-FC(IgG1) | P308362 | Receptor binding domain | 101.58 | >85.0 |

# Kinetics Result

**Work flow :**

1. Instrument: GATOR (ProbeLife)
2. Probe: ProA (LN 11062401)
3. Immobilization of ligands to probe: The antibody was diluted to 15 nM with Q buffer, The antibody coupling experiment is carried out .
4. Dynamic parameter setting： Adjust antigen to 400 nM with Q buffer, after diluting to 200, 100 nM,  the  combination time to 120 s, the dissociation time to 180 s, the default reaction temperature to  30℃, and then detection.
5. Result criteria： R2 > 0.95, system adaptability passed, and data is authentic.

| Number | Construct ID | Protein Number | ACE2-His (P308410 ) | | | | |
|---|---|---|---|---|---|---|---|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 1 | RBD-mut1-FC(IgG1) | P308333 | 3.20E-07 | 1.80E+05 | 5.77E-02 | 0.9981 | 0.8364 |
| 2 | RBD-mut2-FC(IgG1) | P308334 | N/A | N/A | N/A | N/A | N/A |
| 3 | RBD-mut3-FC(IgG1) | P308335 | N/A | N/A | N/A | N/A | N/A |
| 4 | RBD-mut4-FC(IgG1) | P308336 | N/A | N/A | N/A | N/A | N/A |
| 5 | RBD-mut5-FC(IgG1) | P308337 | N/A | N/A | N/A | N/A | N/A |
| 6 | RBD-mut6-FC(IgG1) | P308338 | N/A | N/A | N/A | N/A | N/A |
| 7 | RBD-mut7-FC(IgG1) | P308339 | N/A | N/A | N/A | N/A | N/A |
| 8 | RBD-mut8-FC(IgG1) | P308340 | N/A | N/A | N/A | N/A | N/A |
| 9 | RBD-mut9-FC(IgG1) | P308341 | N/A | N/A | N/A | N/A | N/A |

**KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting**

**Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).**

# Kinetics Result

| Number | Sample ID | Protein Number | ACE2-His (P308410 ) | | | | |
|---|---|---|---|---|---|---|---|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 10 | RBD-mut10-FC(IgG1) | P308342 | N/A | N/A | N/A | N/A | N/A |
| 11 | RBD-mut11-FC(IgG1) | P308343 | N/A | N/A | N/A | N/A | N/A |
| 12 | RBD-mut12-FC(IgG1) | P308344 | N/A | N/A | N/A | N/A | N/A |
| 13 | RBD-mut13-FC(IgG1) | P308345 | N/A | N/A | N/A | N/A | N/A |
| 14 | RBD-mut14-FC(IgG1) | P308346 | N/A | N/A | N/A | N/A | N/A |
| 15 | RBD-mut15-FC(IgG1) | P308347 | N/A | N/A | N/A | N/A | N/A |
| 16 | RBD-mut16-FC(IgG1) | P308348 | N/A | N/A | N/A | N/A | N/A |
| 17 | RBD-mut17-FC(IgG1) | P308349 | N/A | N/A | N/A | N/A | N/A |
| 18 | RBD-mut18-FC(IgG1) | P308350 | N/A | N/A | N/A | N/A | N/A |
| 19 | RBD-mut19-FC(IgG1) | P308351 | 6.54E-08 | 1.29E+05 | 8.43E-03 | 0.9979 | 1.0361 |
| 20 | RBD-mut20-FC(IgG1) | P308352 | 6.31E-08 | 1.10E+05 | 6.93E-03 | 0.9959 | 1.4676 |
| 21 | RBD-mut21-FC(IgG1) | P308353 | N/A | N/A | N/A | N/A | N/A |
| 22 | RBD-mut22-FC(IgG1) | P308354 | 2.32E-07 | 2.64E+05 | 6.13E-02 | 0.9976 | 0.9049 |
| 23 | RBD-mut23-FC(IgG1) | P308355 | N/A | N/A | N/A | N/A | N/A |

KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting

Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).

# Kinetics Result

| Number | Sample ID | Protein number | ACE2-His (P308410 ) | | | | |
|---|---|---|---|---|---|---|---|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 24 | RBD-mut24-FC(IgG1) | P308356 | N/A | N/A | N/A | N/A | N/A |
| 25 | RBD-mut25-FC(IgG1) | P308357 | N/A | N/A | N/A | N/A | N/A |
| 26 | RBD-mut26-FC(IgG1) | P308358 | N/A | N/A | N/A | N/A | N/A |
| 27 | RBD-mut27-FC(IgG1) | P308359 | N/A | N/A | N/A | N/A | N/A |
| 28 | RBD-mut28-FC(IgG1) | P308360 | N/A | N/A | N/A | N/A | N/A |
| 29 | RBD-mut29-FC(IgG1) | P308361 | 2.82E-07 | 1.90E+05 | 5.37E-02 | 0.9942 | 1.0686 |
| 30 | RBD-mut31-FC(IgG1) | P308363 | 1.28E-07 | 2.76E+05 | 3.53E-02 | 0.9942 | 0.867 |
| 31 | RBD-mut32-FC(IgG1) | P308364 | 1.17E-07 | 2.58E+05 | 3.01E-02 | 0.9918 | 1.0486 |
| 32 | RBD-mut33-FC(IgG1) | P308365 | N/A | N/A | N/A | N/A | N/A |
| 33 | RBD-mut34-FC(IgG1) | P308366 | 1.43E-07 | 2.18E+05 | 3.12E-02 | 0.9946 | 1.2666 |
| 34 | RBD-mut35-FC(IgG1) | P308367 | 1.32E-07 | 2.36E+05 | 3.10E-02 | 0.9925 | 1.1653 |
| 35 | RBD-mut36-FC(IgG1) | P308368 | 1.17E-07 | 2.04E+05 | 2.39E-02 | 0.9881 | 1.0267 |
| 36 | RBD-mut37-FC(IgG1) | P308369 | 2.42E-07 | 2.20E+05 | 5.32E-02 | 0.9944 | 0.926 |

KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting

Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).

# Kinetics Result

| Number | Sample ID | Protein number | ACE2-His (P308410 ) | | | | |
|---|---|---|---|---|---|---|---|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 37 | RBD-mut38-FC(IgG1) | P308370 | N/A | N/A | N/A | N/A | N/A |
| 38 | RBD-mut39-FC(IgG1) | P308371 | N/A | N/A | N/A | N/A | N/A |
| 39 | RBD-mut40-FC(IgG1) | P308372 | 2.71E-07 | 1.79E+05 | 4.86E-02 | 0.9976 | 1.0881 |
| 40 | RBD-mut41-FC(IgG1) | P308373 | N/A | N/A | N/A | N/A | N/A |
| 41 | RBD-mut42-FC(IgG1) | P308374 | N/A | N/A | N/A | N/A | N/A |
| 42 | RBD-mut43-FC(IgG1) | P308375 | N/A | N/A | N/A | N/A | N/A |
| 43 | RBD-mut44-FC(IgG1) | P308376 | N/A | N/A | N/A | N/A | N/A |
| 44 | RBD-mut45-FC(IgG1) | P308377 | N/A | N/A | N/A | N/A | N/A |
| 45 | RBD-mut46-FC(IgG1) | P308378 | N/A | N/A | N/A | N/A | N/A |
| 46 | RBD-mut47-FC(IgG1) | P308379 | N/A | N/A | N/A | N/A | N/A |
| 47 | RBD-mut48-FC(IgG1) | P308380 | N/A | N/A | N/A | N/A | N/A |
| 48 | RBD-mut49-FC(IgG1) | P308381 | N/A | N/A | N/A | N/A | N/A |
| 49 | RBD-mut50-FC(IgG1) | P308382 | N/A | N/A | N/A | N/A | N/A |
| 50 | RBD-mut51-FC(IgG1) | P308383 | N/A | N/A | N/A | N/A | N/A |

KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting

Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).

# Kinetics Result

| Number | Sample ID | Protein number | ACE2-His (P308410 ) | | | | |
|--------|-----------|----------------|---------|-----------|----------|----------|----------|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 51 | RBD-mut52-FC(IgG1) | P308384 | N/A | N/A | N/A | N/A | N/A |
| 52 | RBD-mut53-FC(IgG1) | P308385 | N/A | N/A | N/A | N/A | N/A |
| 53 | RBD-mut54-FC(IgG1) | P308386 | N/A | N/A | N/A | N/A | N/A |
| 54 | RBD-mut55-FC(IgG1) | P308387 | N/A | N/A | N/A | N/A | N/A |
| 55 | RBD-mut56-FC(IgG1) | P308388 | 2.43E-07 | 2.09E+05 | 5.08E-02 | 0.9951 | 1.0592 |
| 56 | RBD-mut57-FC(IgG1) | P308389 | 2.37E-07 | 2.02E+05 | 4.79E-02 | 0.9955 | 1.1715 |
| 57 | RBD-mut58-FC(IgG1) | P308390 | 2.16E-07 | 2.26E+05 | 4.90E-02 | 0.989 | 0.9693 |
| 58 | RBD-mut59-FC(IgG1) | P308391 | N/A | N/A | N/A | N/A | N/A |
| 59 | RBD-mut60-FC(IgG1) | P308392 | 2.43E-08 | 1.46E+05 | 3.54E-03 | 0.9862 | 1.6085 |
| 60 | RBD-mut61-FC(IgG1) | P308393 | N/A | N/A | N/A | N/A | N/A |
| 61 | RBD-mut62-FC(IgG1) | P308394 | 2.43E-08 | 2.01E+05 | 4.90E-03 | 0.9864 | 1.3462 |
| 62 | RBD-mut63-FC(IgG1) | P308395 | N/A | N/A | N/A | N/A | N/A |
| 63 | RBD-mut64-FC(IgG1) | P308396 | 1.24E-07 | 2.34E+05 | 2.89E-02 | 0.9897 | 1.3029 |
| 64 | RBD-mut65-FC(IgG1) | P308397 | 5.46E-08 | 1.28E+05 | 7.01E-03 | 0.9921 | 1.4697 |
| 65 | RBD-mut66-FC(IgG1) | P308398 | 5.39E-08 | 1.32E+05 | 7.11E-03 | 0.9929 | 1.2938 |

KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting

Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).

# Kinetics Result

| Number | Sample ID | Protein number | ACE2-His (P308410 ) | | | | |
|---|---|---|---|---|---|---|---|
| | | | KD (M) | ka(1/Ms) | kd(1/s) | $R^2$ | $R_{max}$ (nm) |
| 66 | RBD-mut67-FC(IgG1) | P308399 | 5.14E-08 | 1.24E+05 | 6.40E-03 | 0.9924 | 1.6548 |
| 67 | RBD-mut68-FC(IgG1) | P308400 | 1.34E-07 | 5.74E+05 | 7.72E-02 | 0.9525 | 0.1651 |
| 68 | RBD-mut69-FC(IgG1) | P308401 | 2.26E-08 | 1.76E+05 | 3.98E-03 | 0.9886 | 1.2357 |
| 69 | RBD-mut70-FC(IgG1) | P308402 | 5.22E-08 | 1.29E+05 | 6.73E-03 | 0.9907 | 1.3488 |
| 70 | RBD-mut71-FC(IgG1) | P308403 | 5.22E-08 | 1.49E+05 | 7.77E-03 | 0.9953 | 1.2501 |
| 71 | RBD-mut72-FC(IgG1) | P308404 | 4.98E-08 | 1.53E+05 | 7.63E-03 | 0.994 | 1.2736 |
| 72 | RBD-mut73-FC(IgG1) | P308405 | 1.22E-07 | 1.47E+05 | 1.79E-02 | 0.9691 | 0.2624 |
| 73 | RBD-mut74-FC(IgG1) | P308406 | N/A | N/A | N/A | N/A | N/A |
| 74 | RBD-mut75-FC(IgG1) | P308407 | N/A | N/A | N/A | N/A | N/A |
| 75 | RBD-mut76-FC(IgG1) | P308408 | 9.29E-07 | 5.31E+04 | 4.93E-02 | 0.9518 | 0.3157 |
| 76 | RBD-mut77-FC(IgG1) | P308409 | 1.11E-07 | 1.52E+05 | 1.69E-02 | 0.9989 | 1.3295 |
| 77 | RBD-mut30-FC(IgG1) | P308362 | 1.52E-07 | 2.22E+05 | 3.37E-02 | 0.9966 | 0.7542 |

KD：dissociation constant/association constant；Ka：association constant；Kd：dissociation constant；R2：Linear fitting constant；Rmax：The highest response value for curve fitting

Please note that the 'Number' in the table does not directly correspond to the 'mut' number in the Sample ID. For example, Number 30 corresponds to a Sample ID of RBD-mut31-FC(IgG1).

## N/A: very weak/no binding

# Sensorgrams
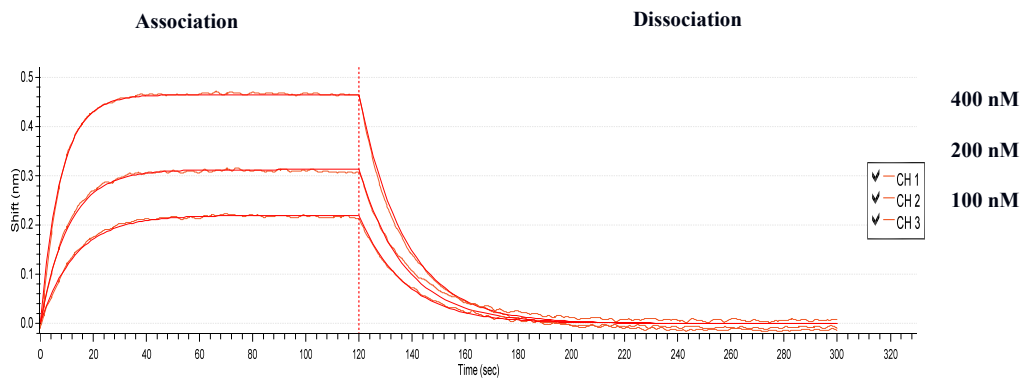


Fig 1.RBD-mut1-FC(IgG1)
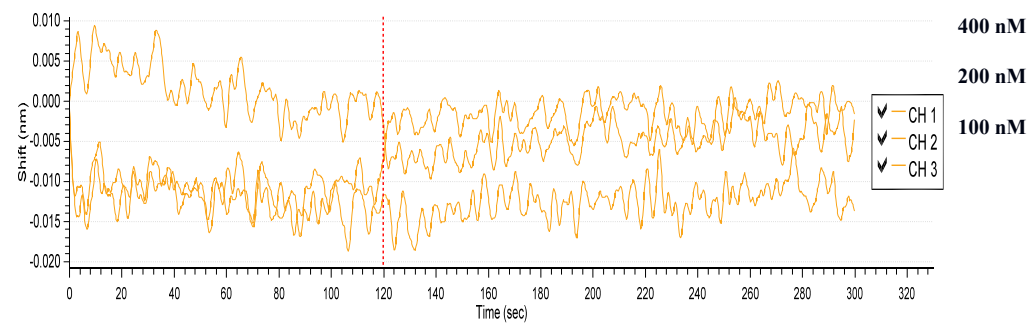


Fig 2. RBD-mut2-FC(IgG1)
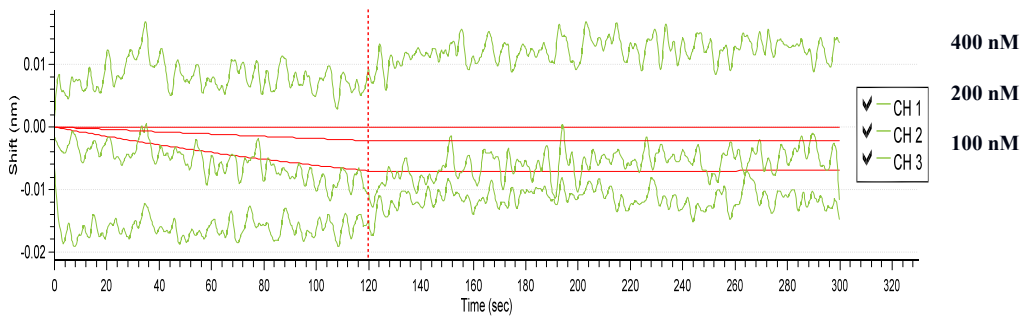


Fig 3. RBD-mut3-FC(IgG1)
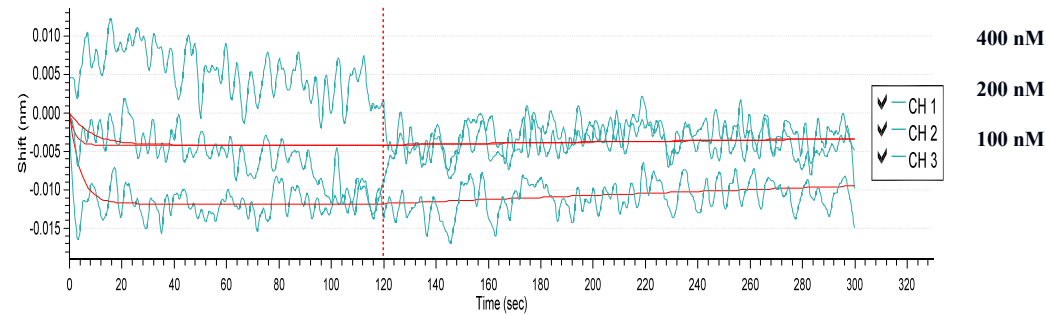


Fig 4. RBD-mut4-FC(IgG1)

# Sensorgrams
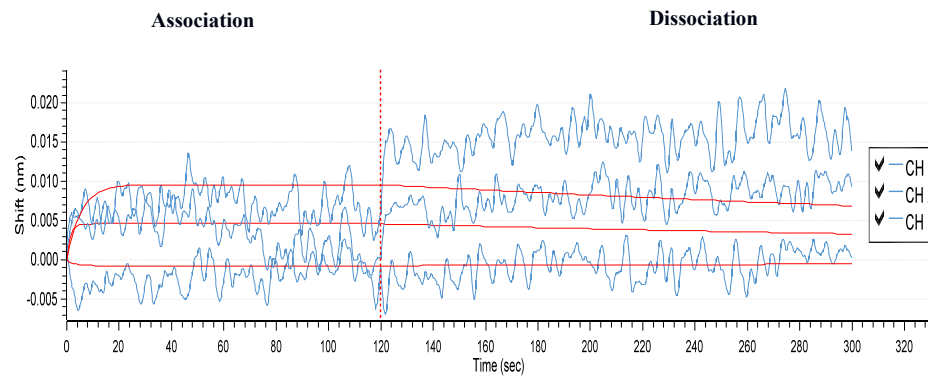


Fig 5. RBD-mut5-FC(IgG1)
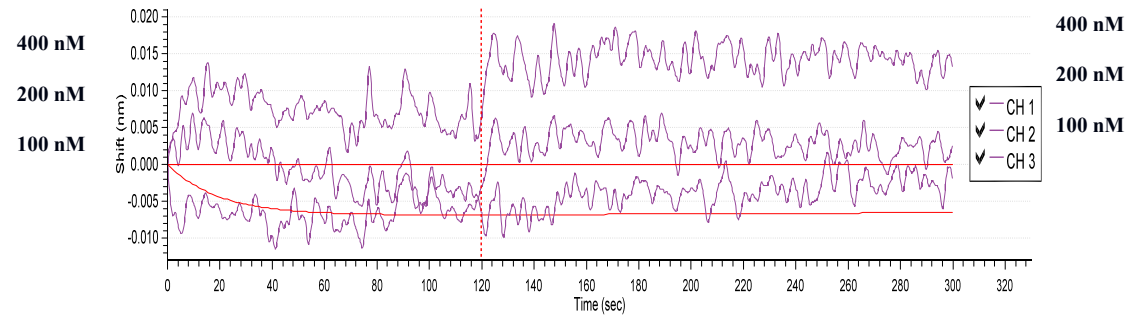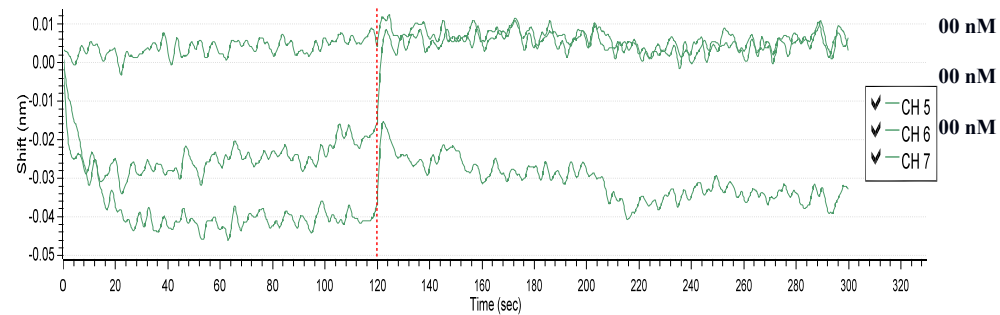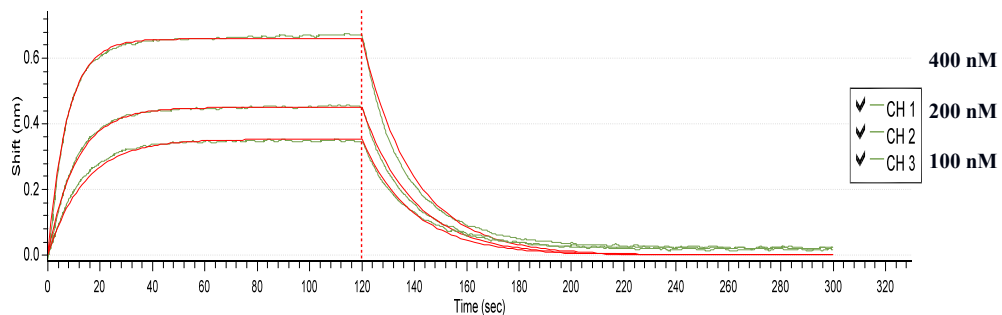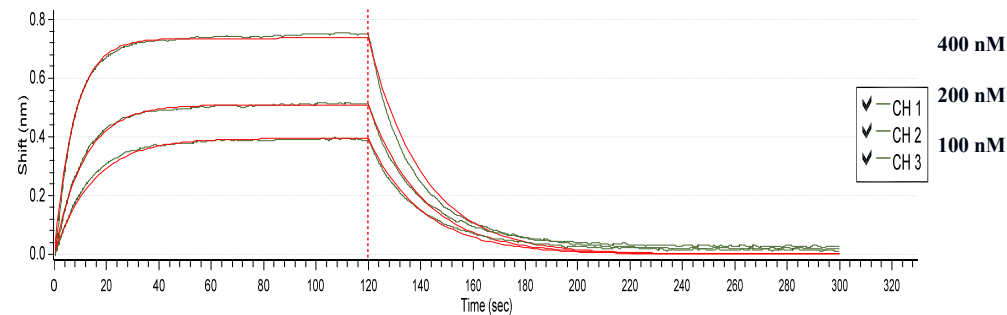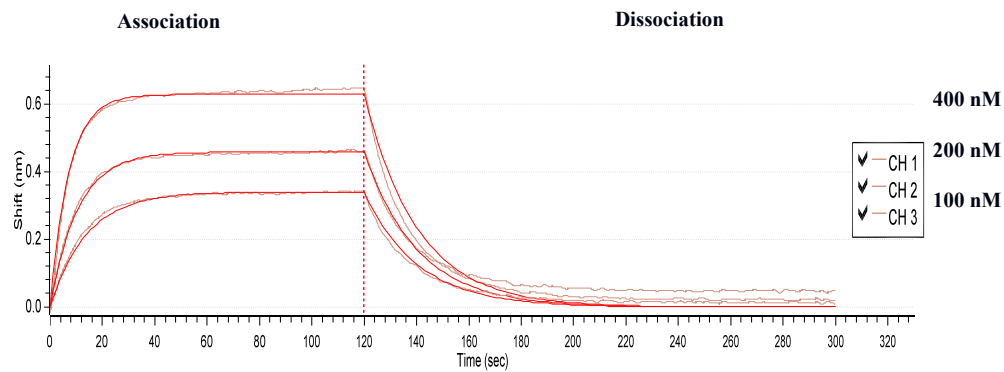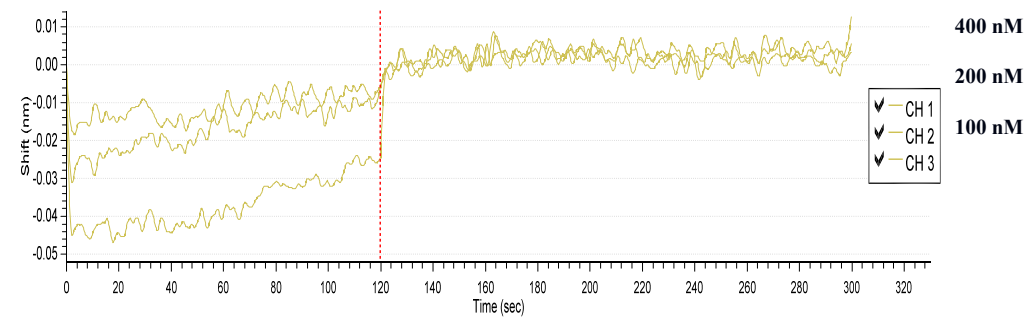


Fig 6. RBD-mut6-FC(IgG1)
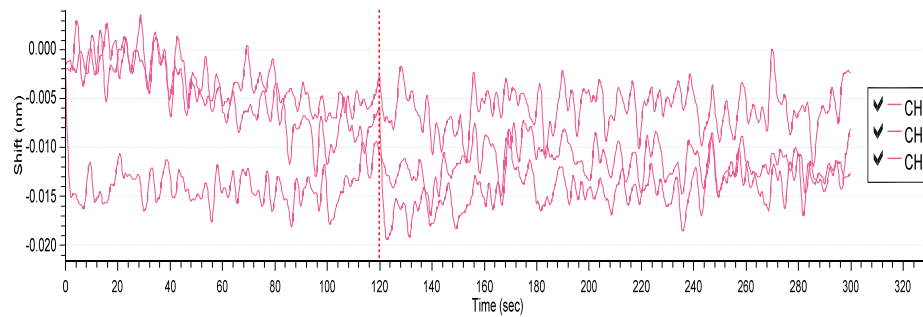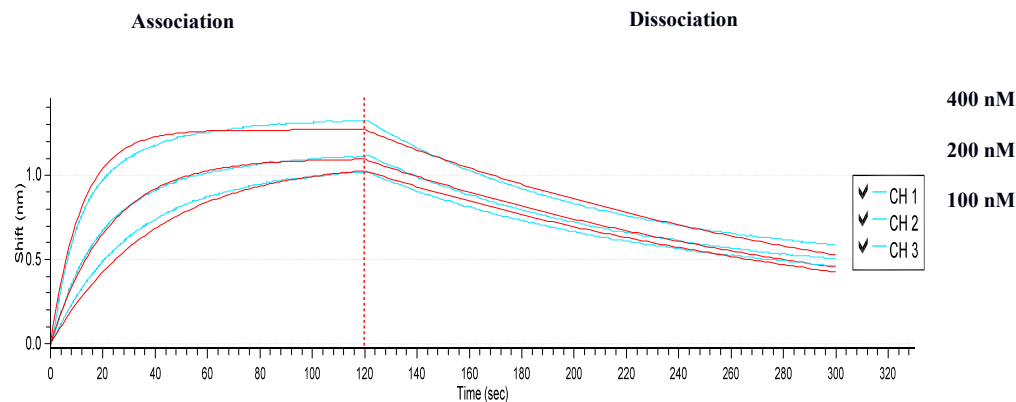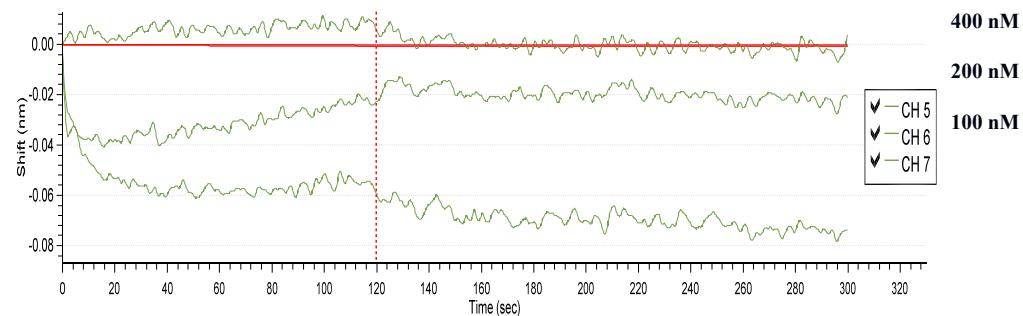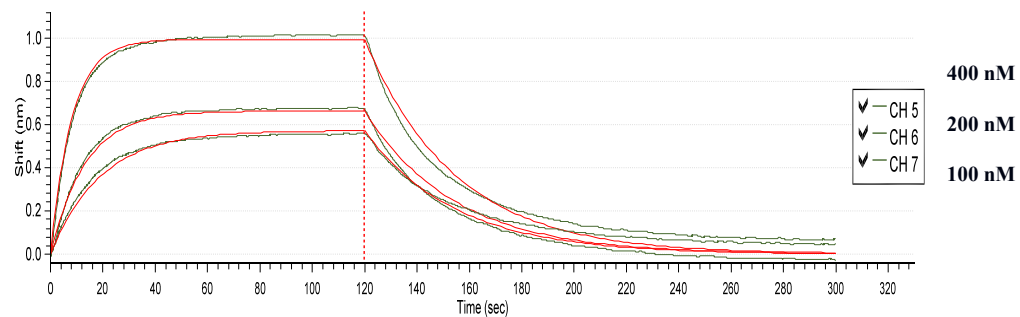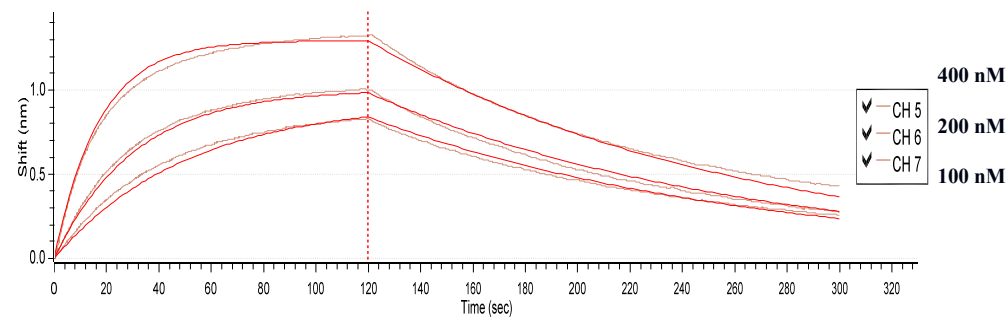


Fig 7. RBD-mut7-FC(IgG1)
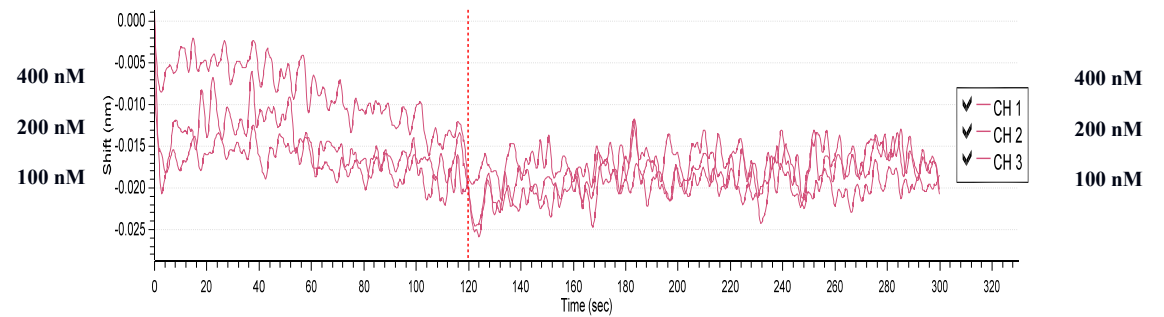


Fig 8. RBD-mut8-FC(IgG1)

# Sensorgrams
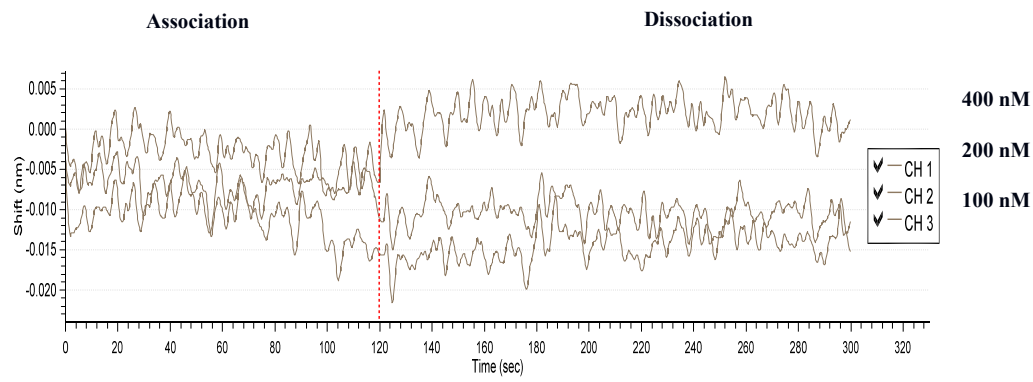


Fig 9. RBD-mut9-FC(IgG1)
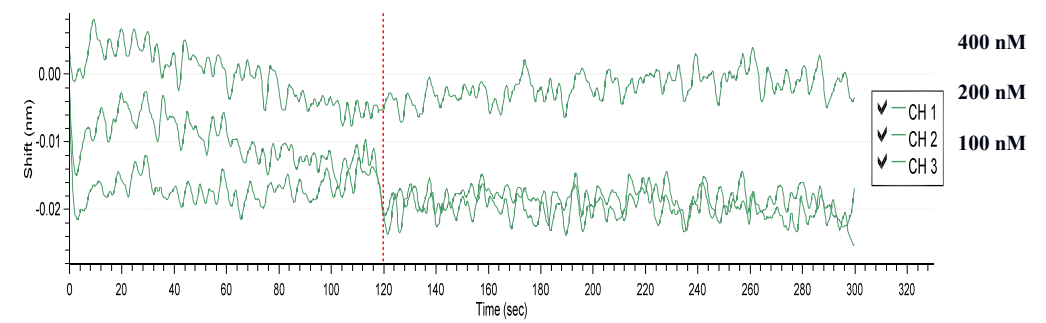


Fig 10. RBD-mut10-FC(IgG1)
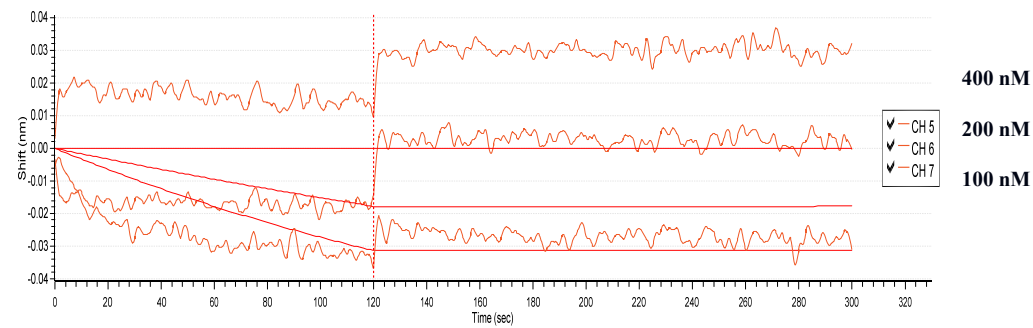


Fig 11. RBD-mut11-FC(IgG1)
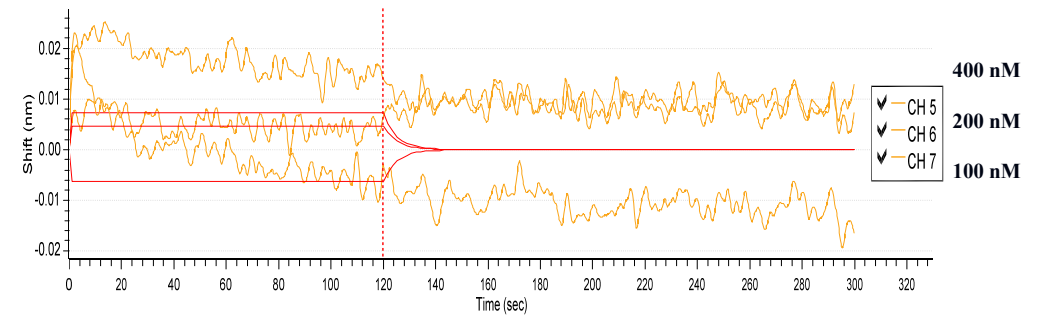


Fig 12. RBD-mut12-FC(IgG1)

# Sensorgrams
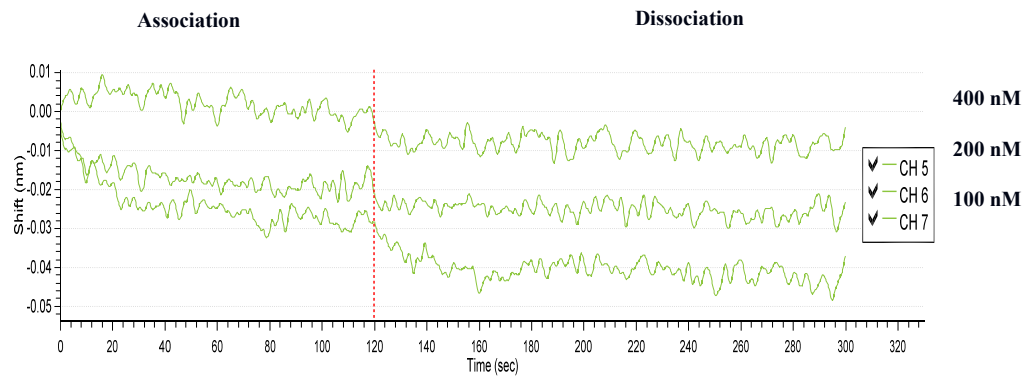


Fig 13. RBD-mut13-FC(IgG1)
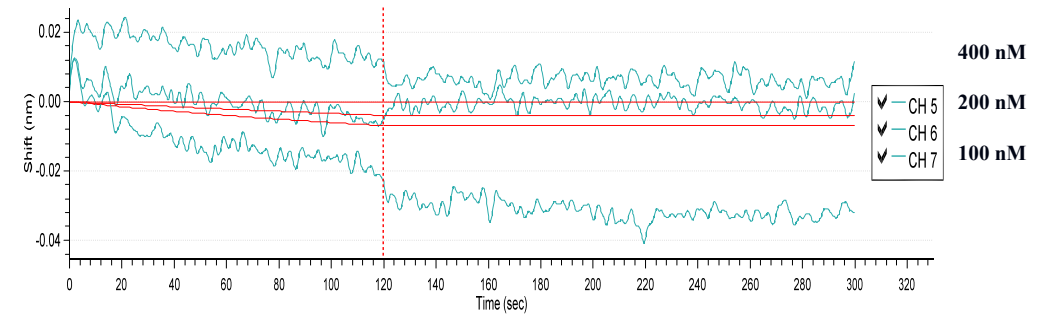


Fig 14. RBD-mut14-FC(IgG1)
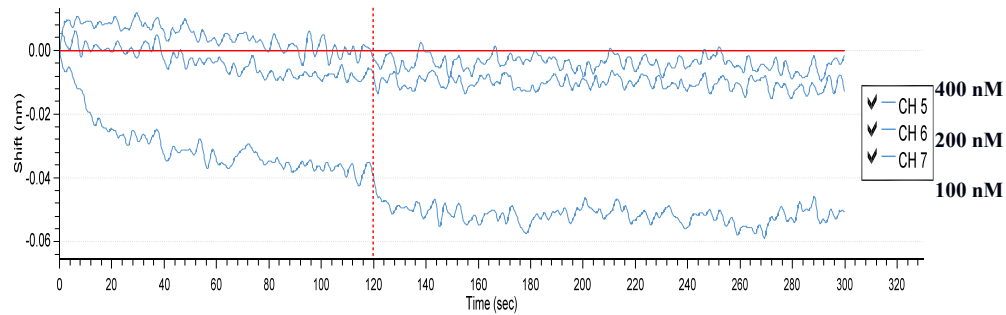


Fig 15. RBD-mut15-FC(IgG1)
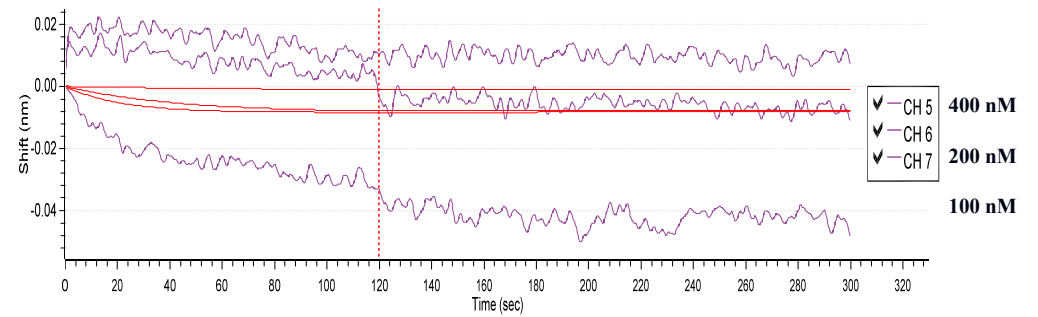


Fig 16. RBD-mut16-FC(IgG

# Sensorgrams
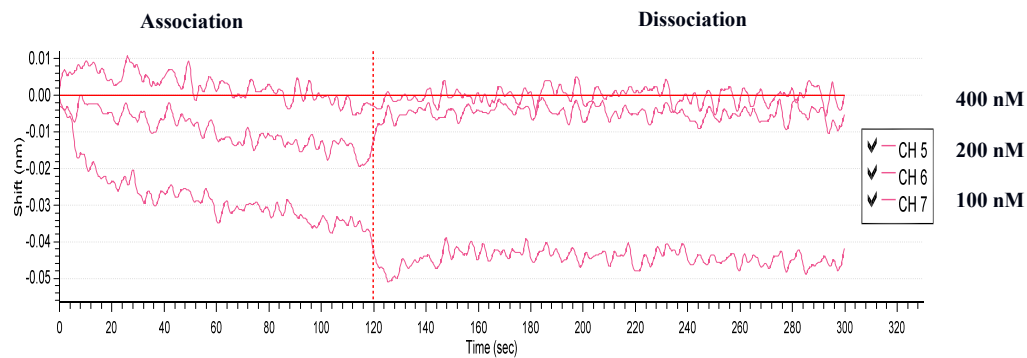


Fig 17. RBD-mut17-FC(IgG1)
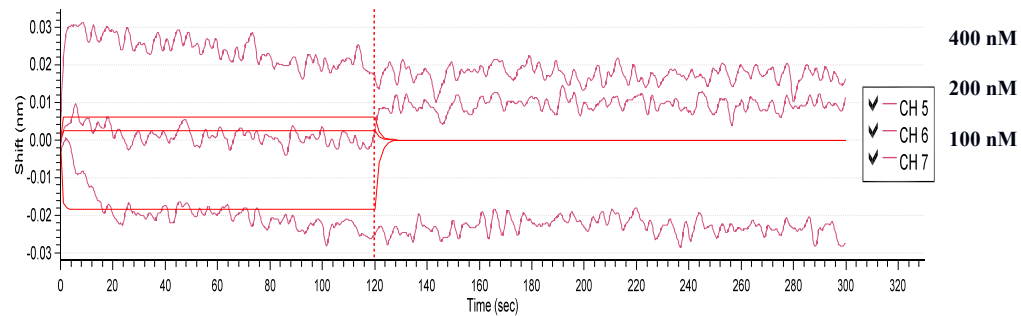


Fig 18. RBD-mut18-FC(IgG1)
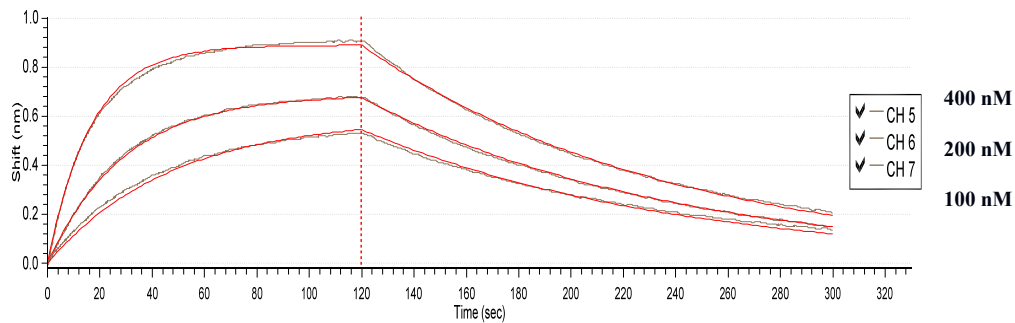


Fig 19. RBD-mut19-FC(IgG1)
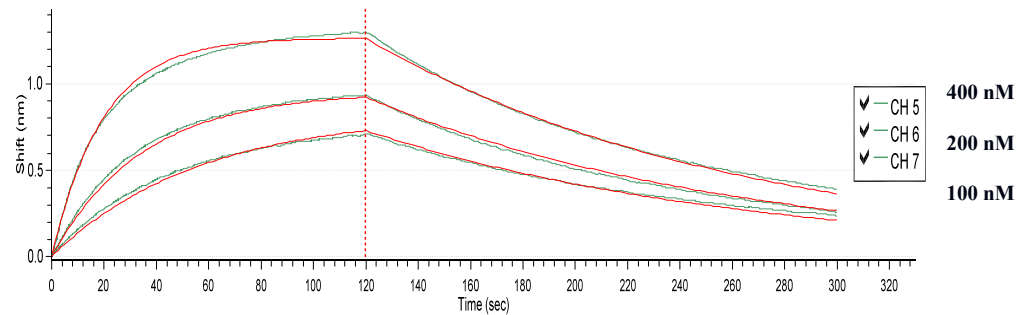


Fig 20. RBD-mut20-FC(IgG1)

# Sensorgrams
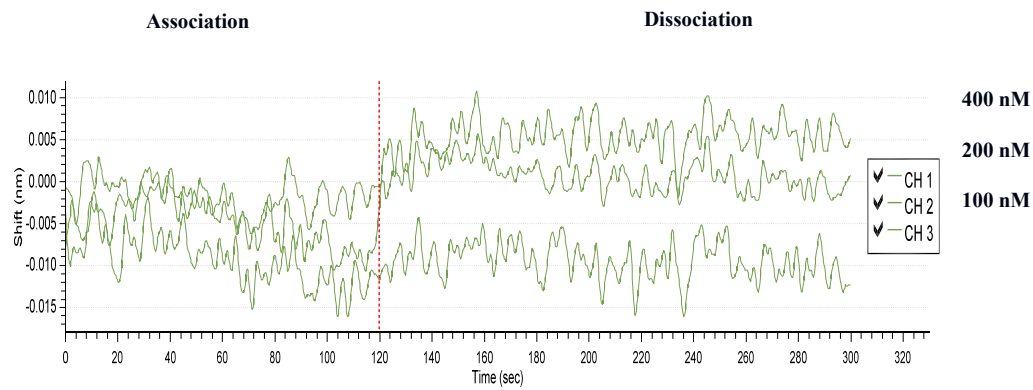


Fig 21. RBD-mut21-FC(IgG1)
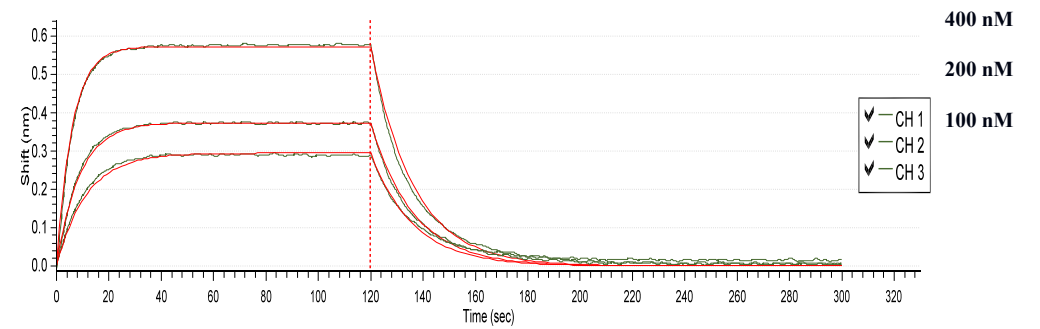


Fig 22. RBD-mut22-FC(IgG1)
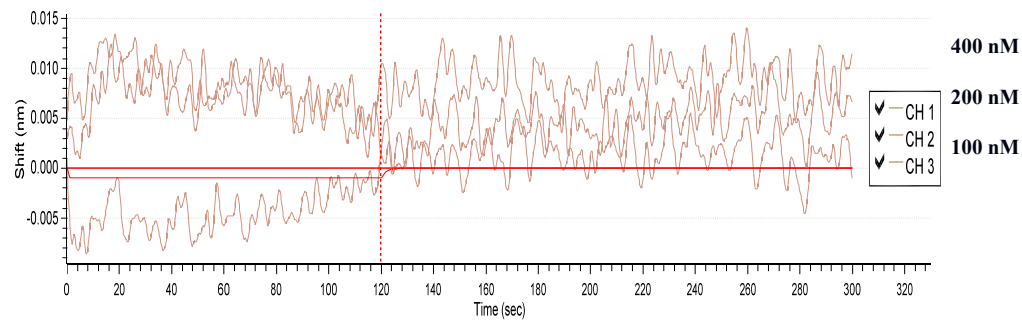


Fig 23. RBD-mut23-FC(IgG1)
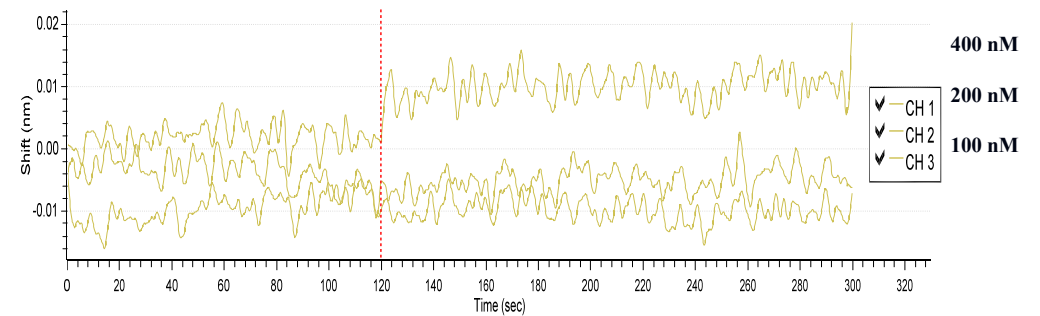


Fig 24. RBD-mut24-FC(IgG1)

# Sensorgrams
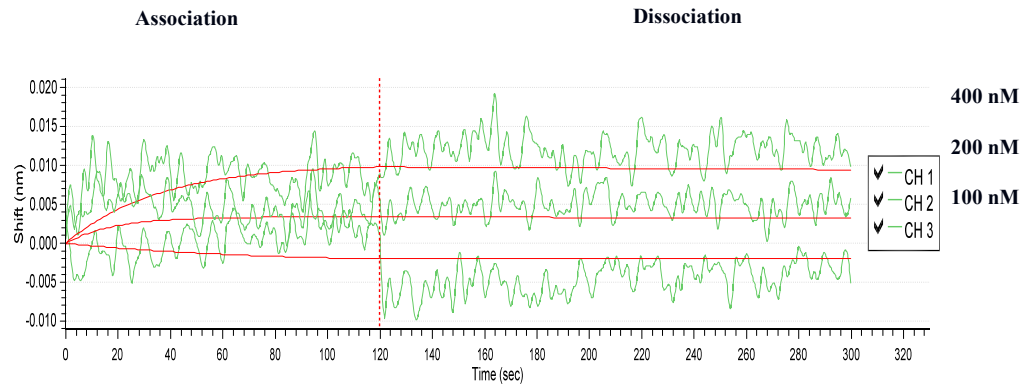


Fig 25. RBD-mut25-FC(IgG1)
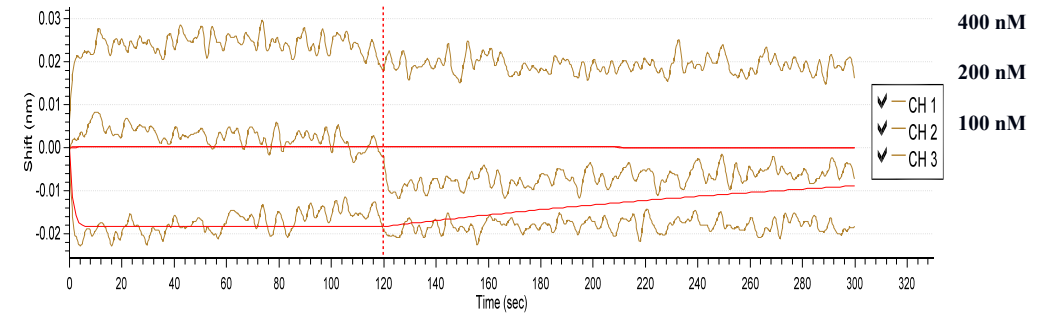


Fig 26. RBD-mut26-FC(IgG1)
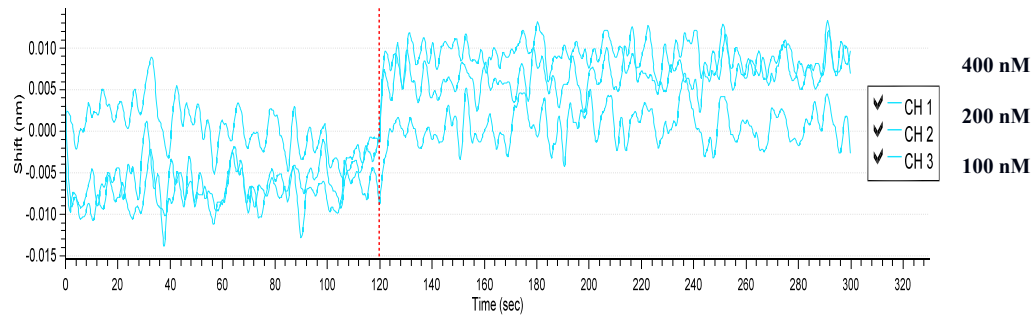


Fig 27. RBD-mut27-FC(IgG1)
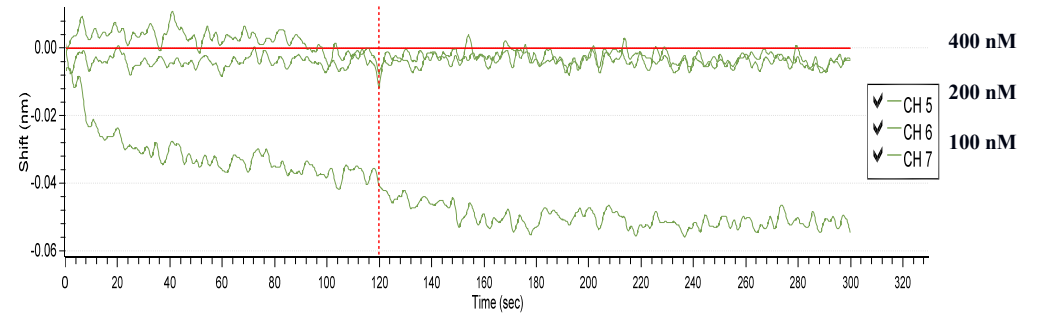


Fig 28. RBD-mut28-FC(IgG1)

# Sensorgrams
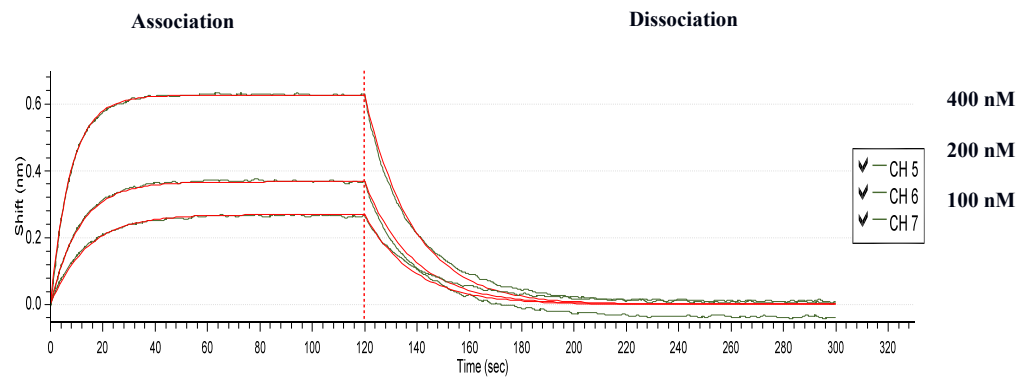


Fig 29. RBD-mut29-FC(IgG1)
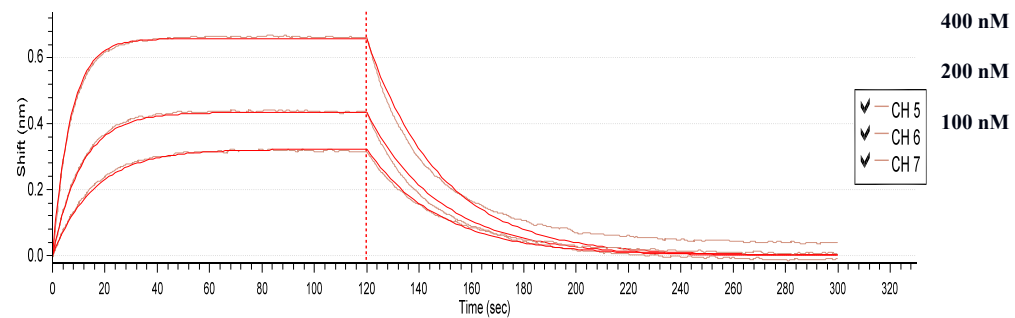


Fig 30. RBD-mut31-FC(IgG1)
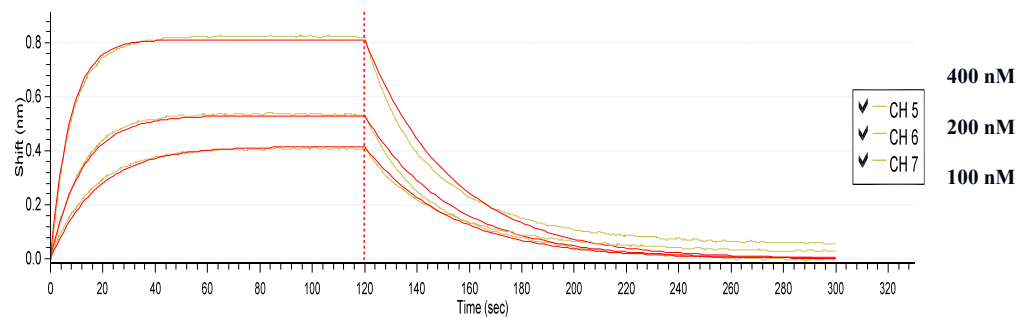


Fig 31. RBD-mut32-FC(IgG1)
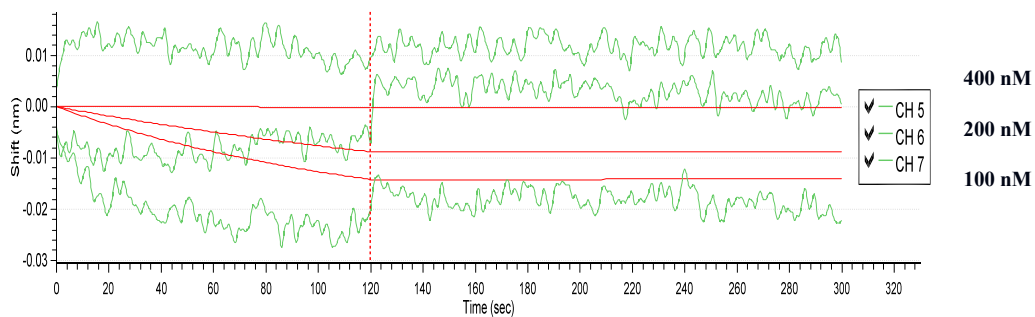


Fig 32. RBD-mut33-FC(IgG1)

# Sensorgrams
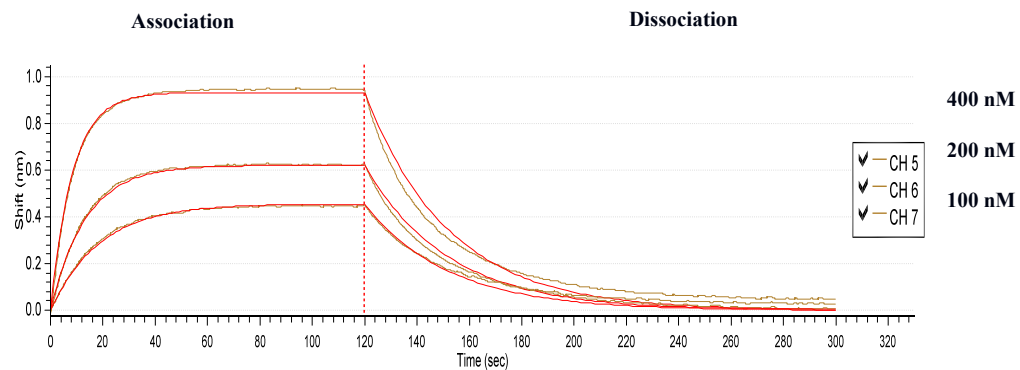


Fig 33. RBD-mut34-FC(IgG1)
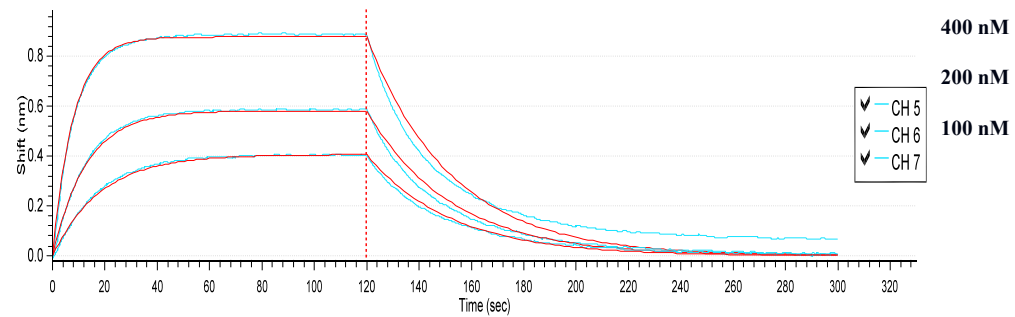


Fig 34. RBD-mut35-FC(IgG1)
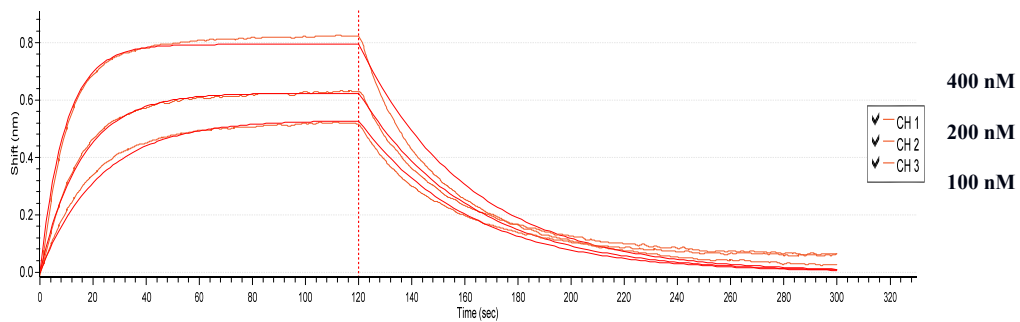


Fig 35. RBD-mut36-FC(IgG1)
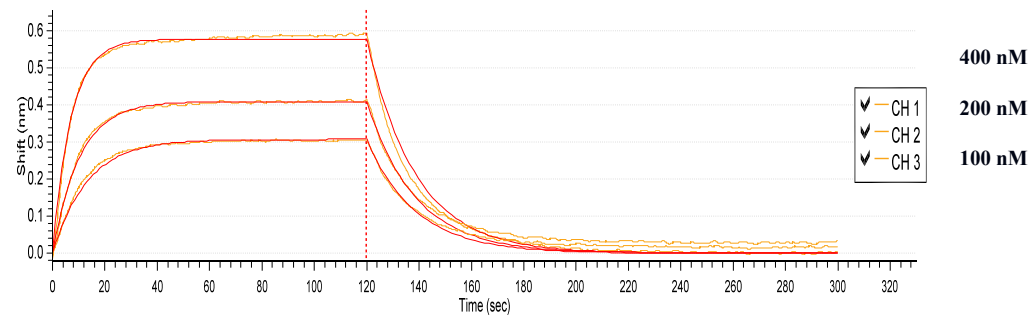


Fig 36. RBD-mut37-FC(IgG1)

# Sensorgrams
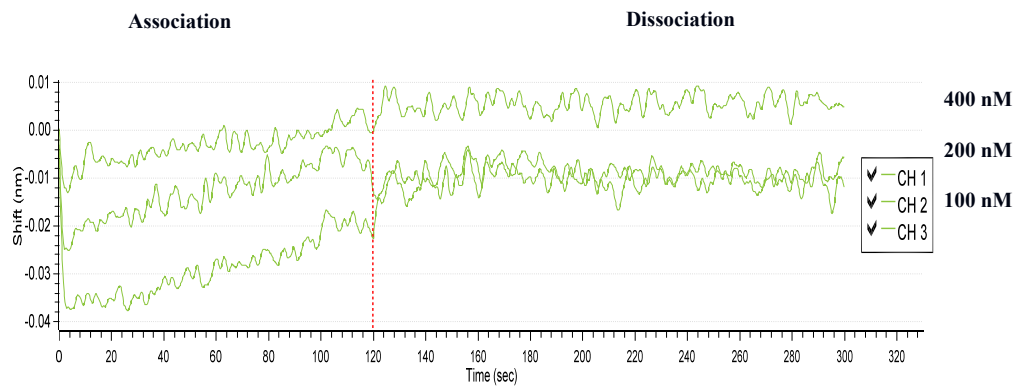


Fig 37. RBD-mut38-FC(IgG1)
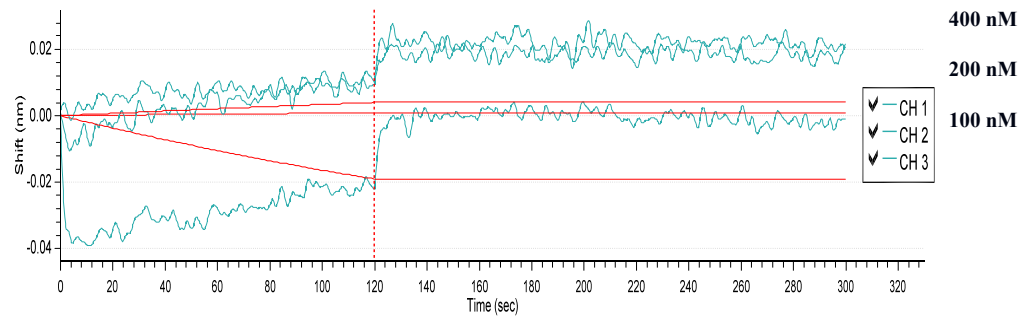


Fig 38. RBD-mut39-FC(IgG1)
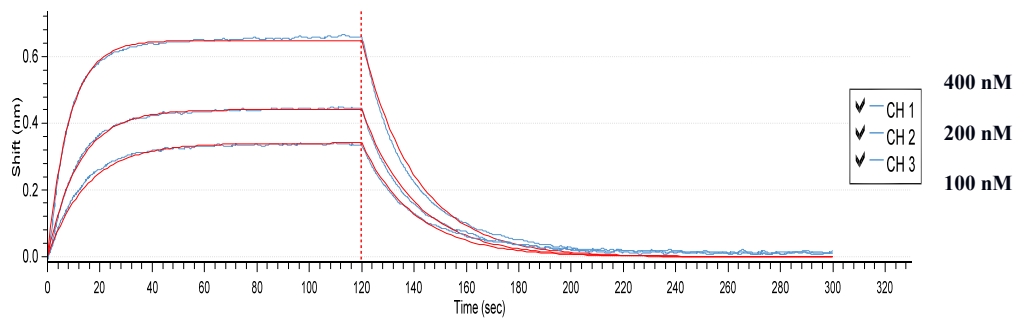


Fig 39. RBD-mut40-FC(IgG1)
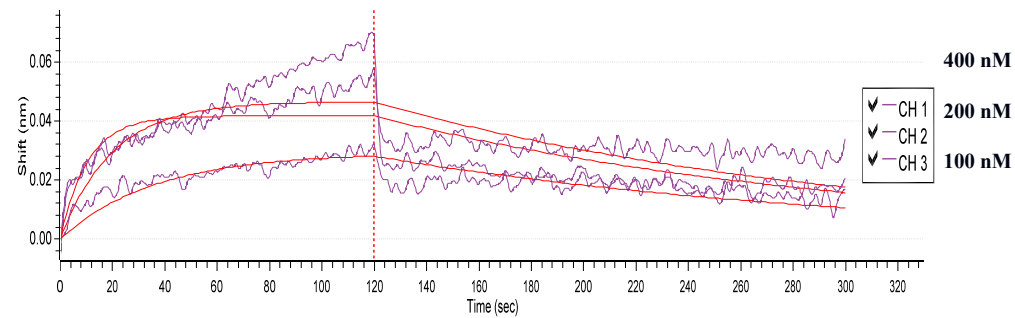


Fig 40. RBD-mut41-FC(IgG1)

# Sensorgrams
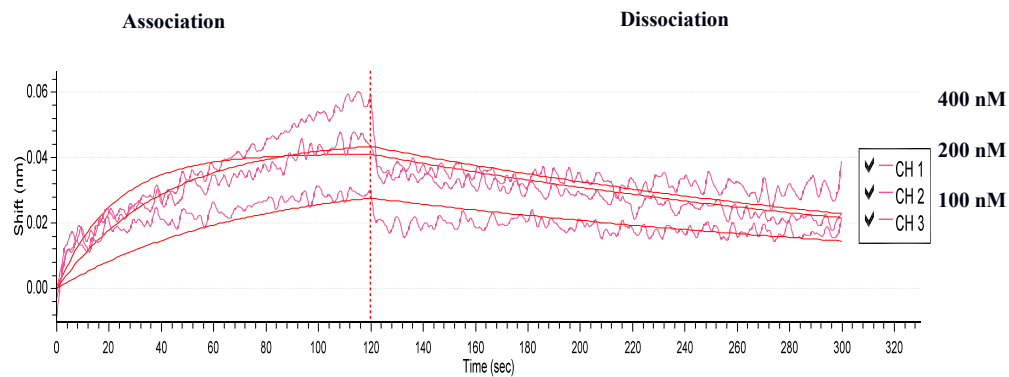


Fig 41. RBD-mut42-FC(IgG1)
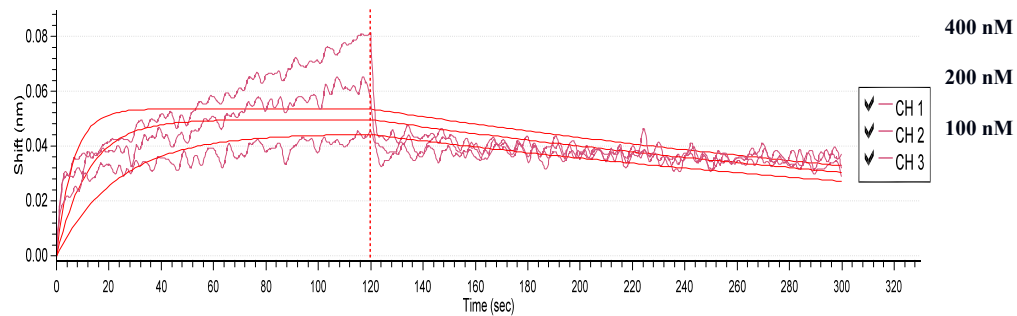


Fig 42. RBD-mut43-FC(IgG1)
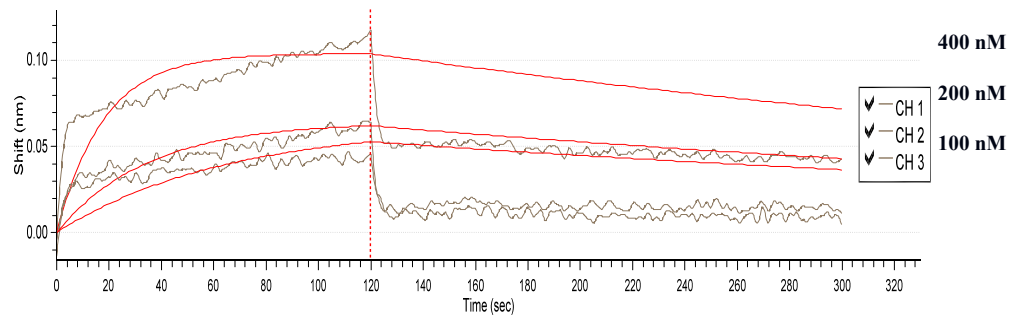


Fig 43. RBD-mut44-FC(IgG1)
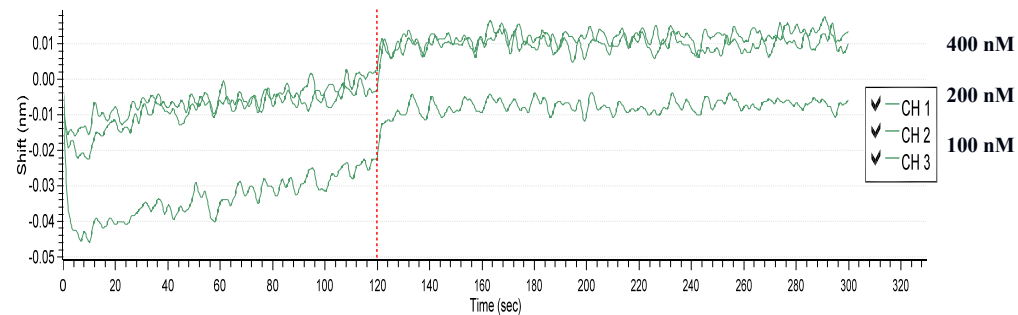


Fig 44. RBD-mut45-FC(IgG1)

# Sensorgrams
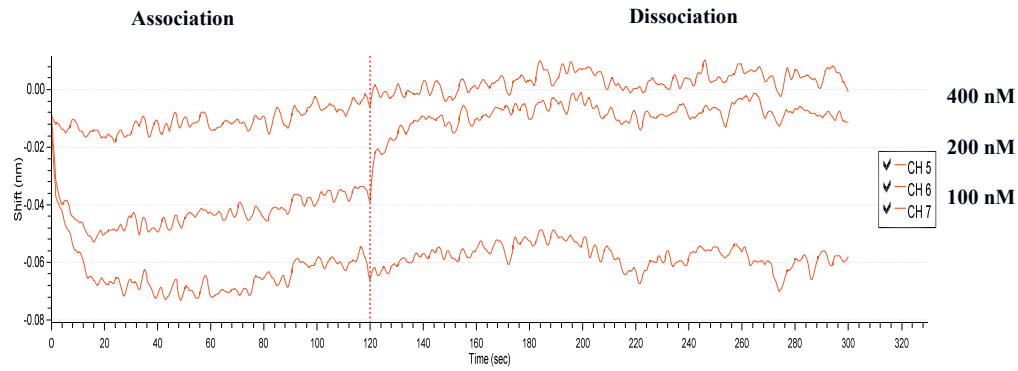


Fig 45. RBD-mut46-FC(IgG1)



Fig 46. RBD-mut47-FC(IgG1)



Fig 47. RBD-mut48-FC(IgG1)
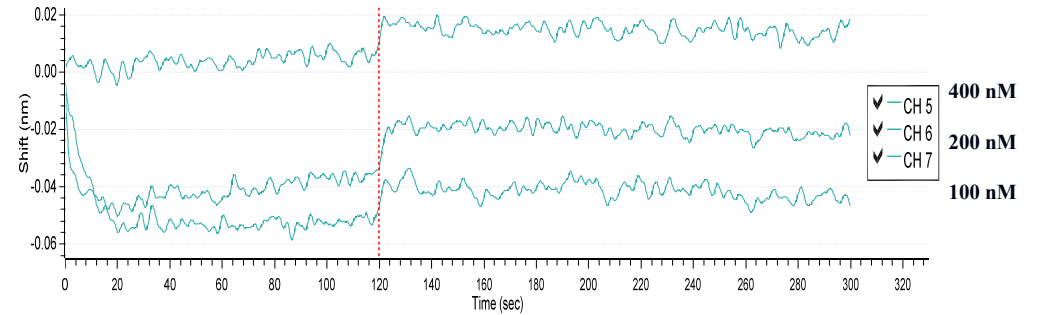


Fig 48. RBD-mut49-FC(IgG1)

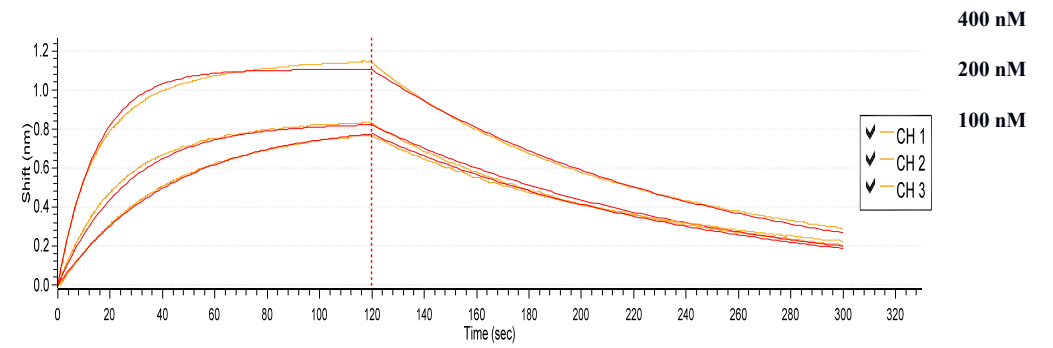# Sensorgrams
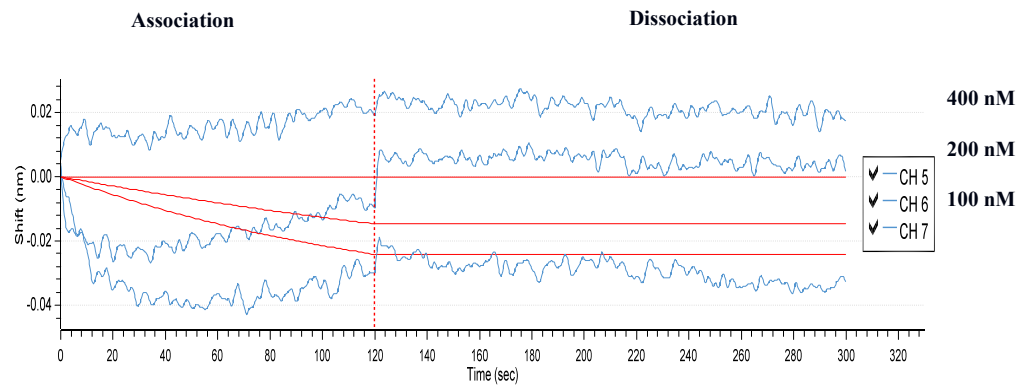


Fig 49. RBD-mut50-FC(IgG1)
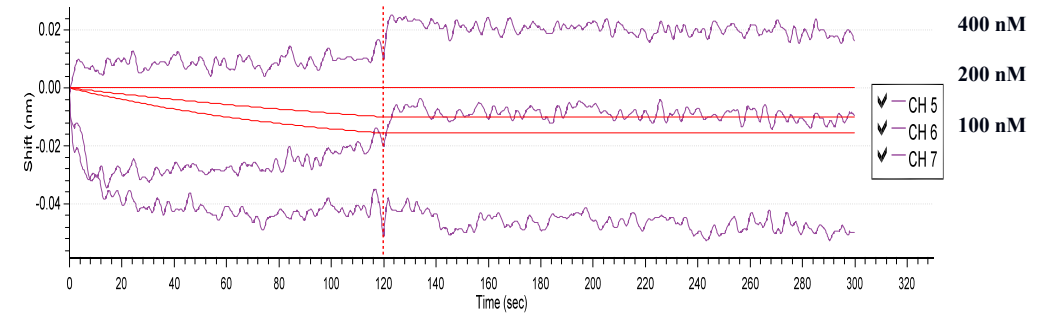


Fig 50. RBD-mut51-FC(IgG1)
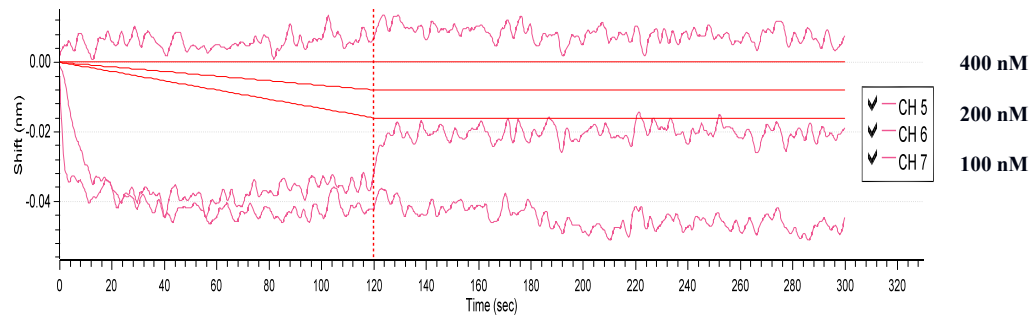


Fig 51. RBD-mut52-FC(IgG1)
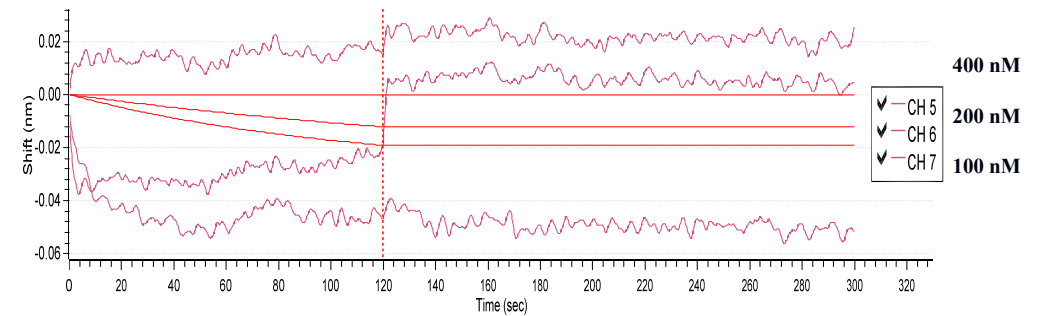


Fig 52. RBD-mut53-FC(IgG1)
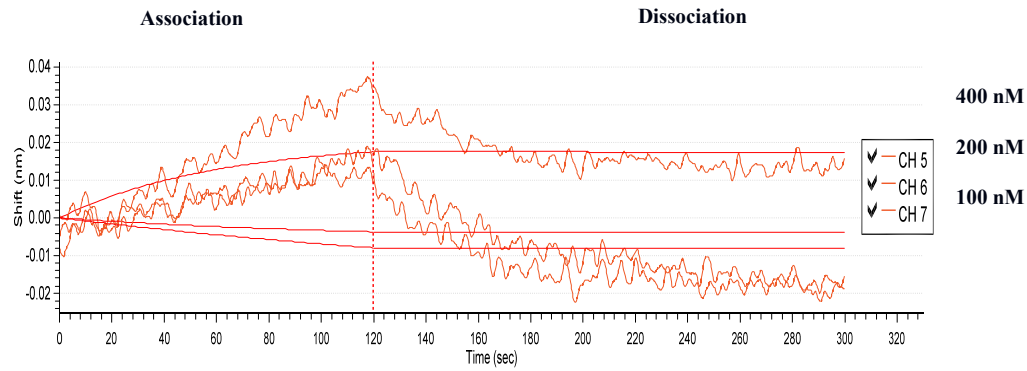
# Sensorgrams
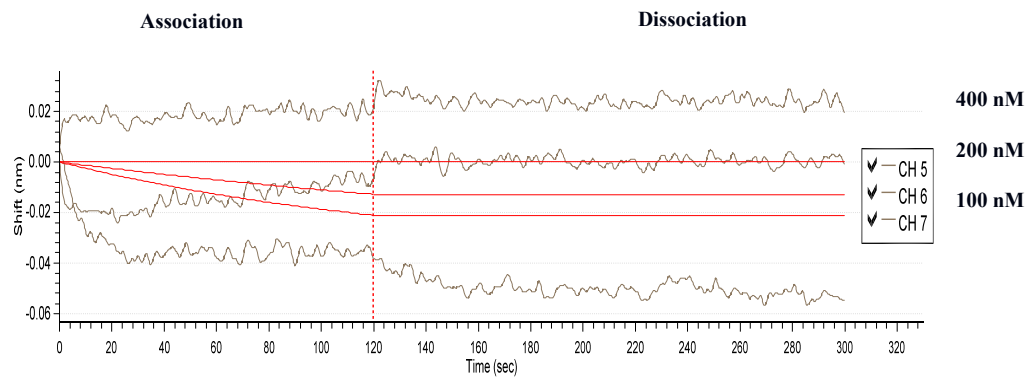


Fig 53. RBD-mut54-FC(IgG1)



Fig 54. RBD-mut55-FC(IgG1)



Fig 55. RBD-mut56-FC(IgG1)



Fig 56. RBD-mut57-FC(IgG1)

# Sensorgrams



Fig 57. RBD-mut58-FC(IgG1)



Fig 58. RBD-mut59-FC(IgG1)
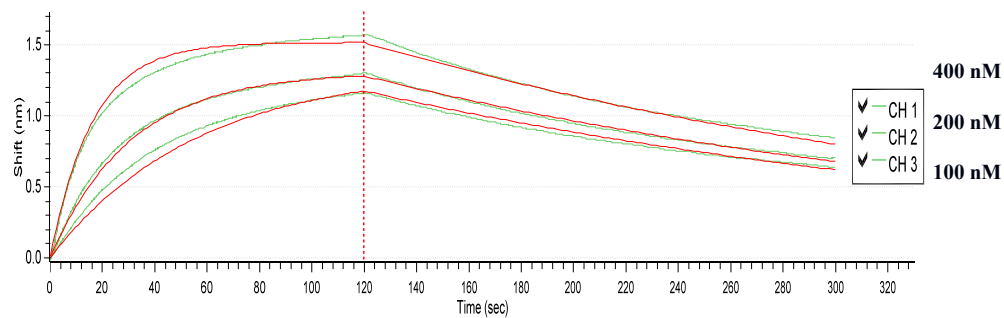


Fig 59. RBD-mut60-FC(IgG1)
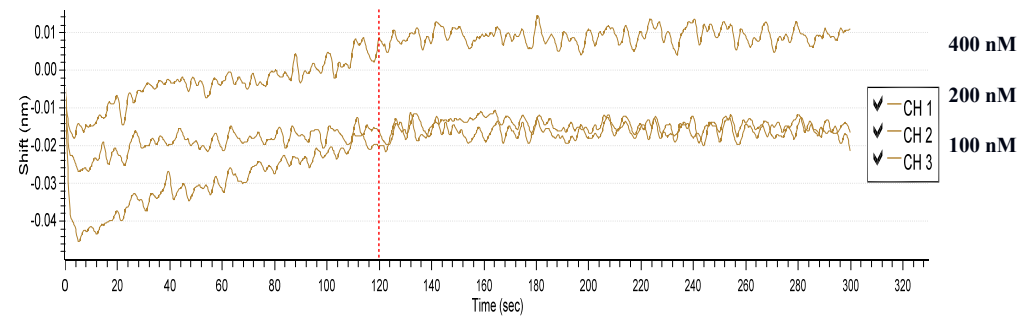


Fig 60. RBD-mut61-FC(IgG1)

# Sensorgrams



Fig 61. RBD-mut62-FC(IgG1)



Fig 62. RBD-mut63-FC(IgG1)



Fig 63. RBD-mut64-FC(IgG1)



Fig 64. RBD-mut65-FC(IgG1)

# Sensorgrams
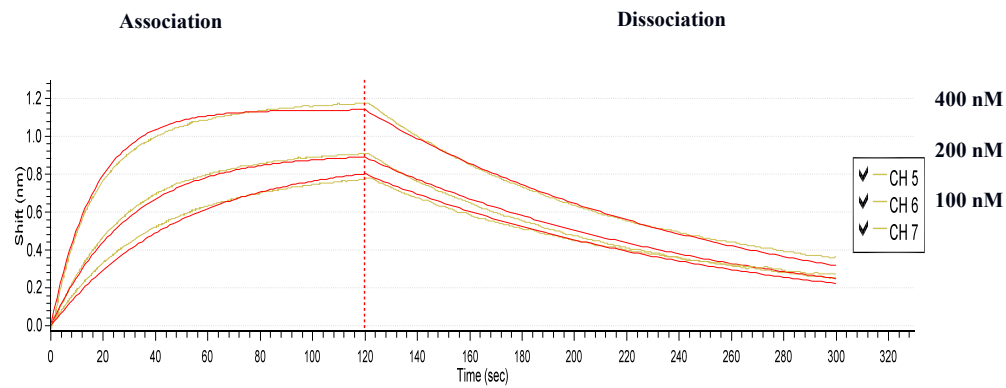


Fig 65. RBD-mut66-FC(IgG1)
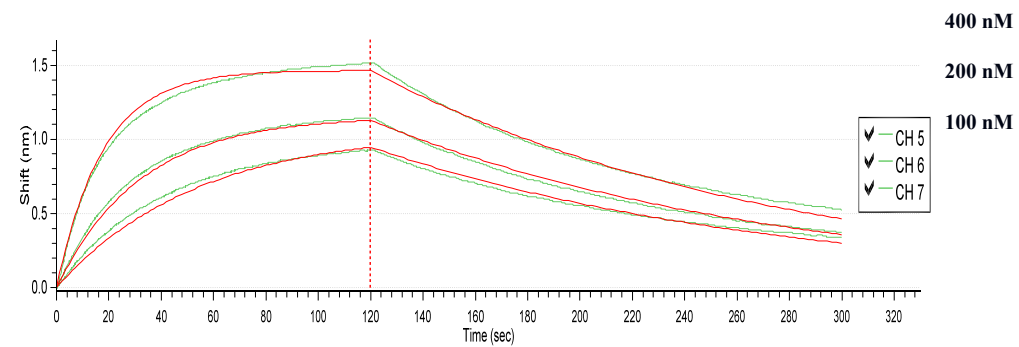


Fig 66. RBD-mut67-FC(IgG1)
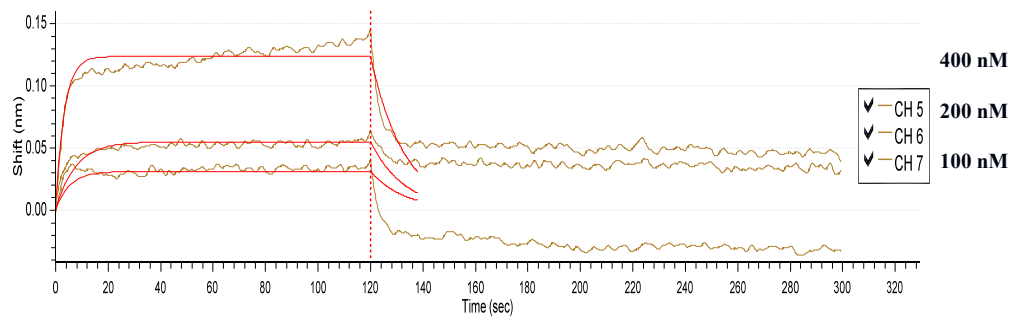


Fig 67. RBD-mut68-FC(IgG1)
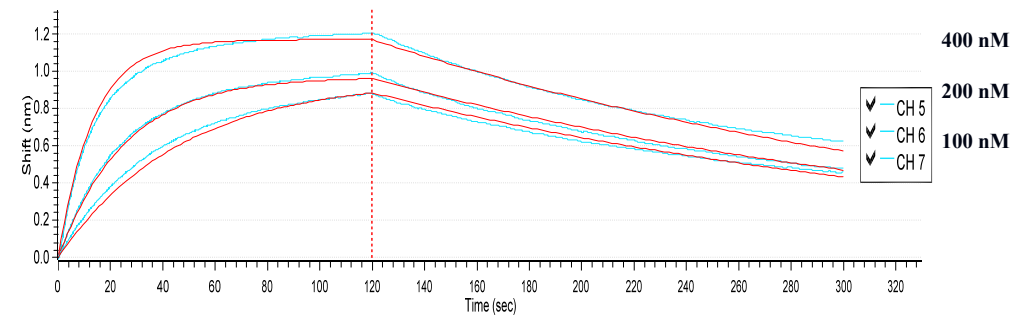


Fig 68. RBD-mut69-FC(IgG1)

# Sensorgrams
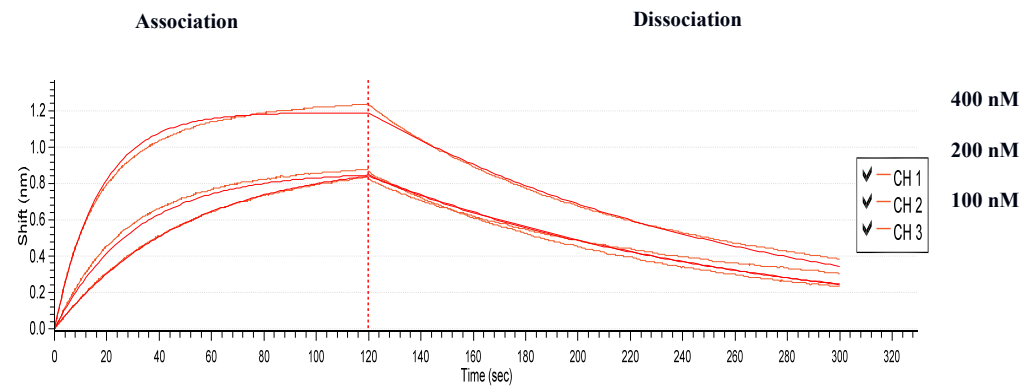


Fig 69. RBD-mut70-FC(IgG1)



Fig 70. RBD-mut71-FC(IgG1)
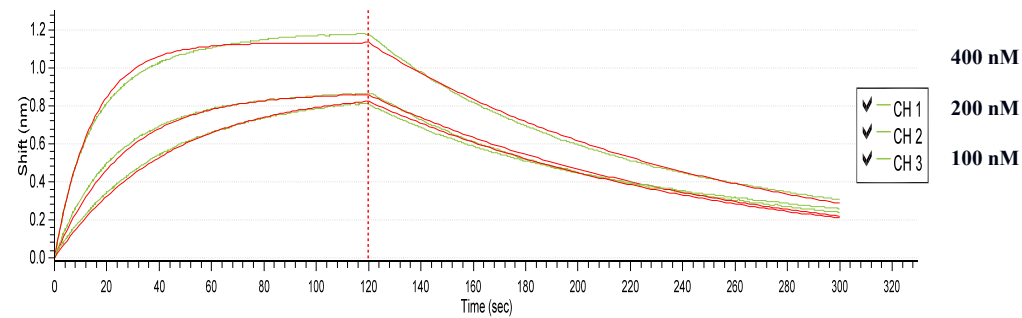


Fig 71. RBD-mut72-FC(IgG1)
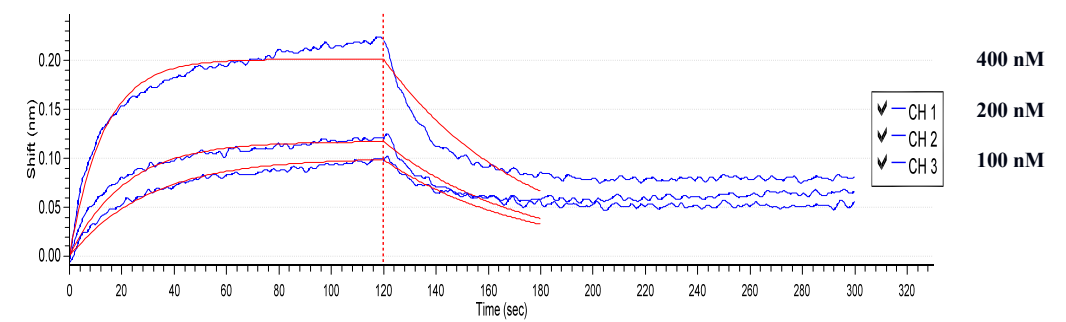


Fig 72. RBD-mut73-FC(IgG1)
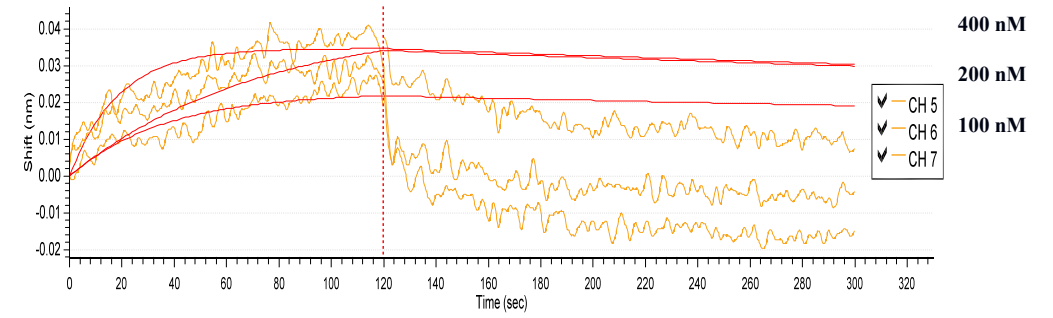
# Sensorgrams



Fig 73. RBD-mut74-FC(IgG1)
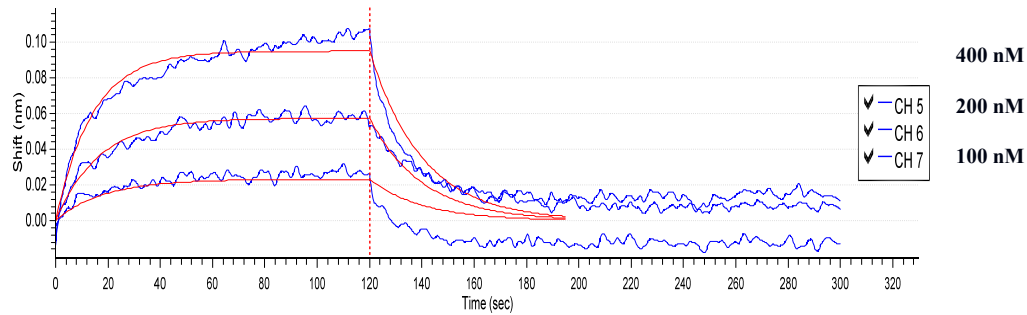


Fig 74. RBD-mut75-FC(IgG1)
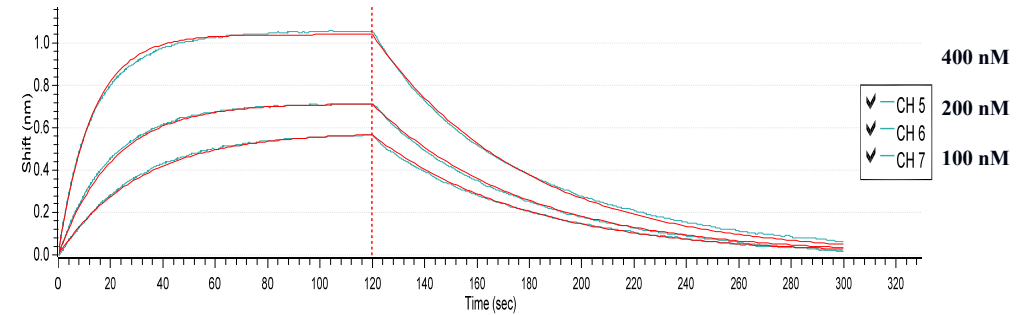


Fig 75. RBD-mut76-FC(IgG1)



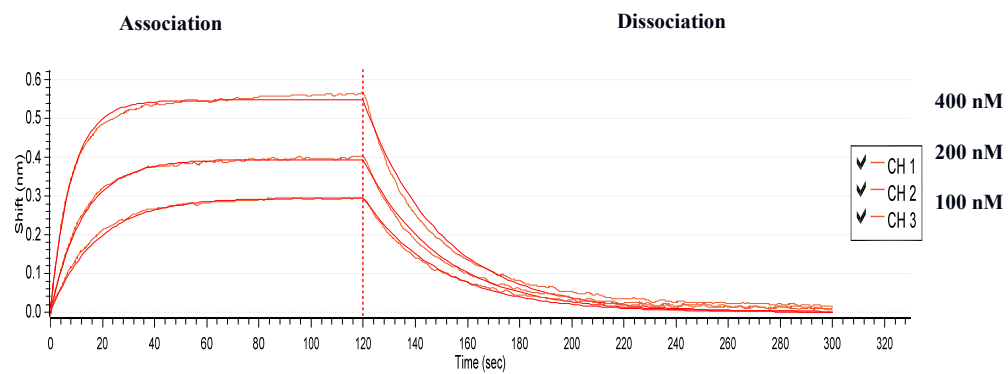Fig 76. RBD-mut77-FC(IgG1)

# Sensorgrams



Fig 77. RBD-mut30-FC(IgG1)

Please note that the 'Fig Number' does not directly correspond to the 'mut number'. For example, Fig 77. corresponds to RBD-mut30-FC(IgG1).