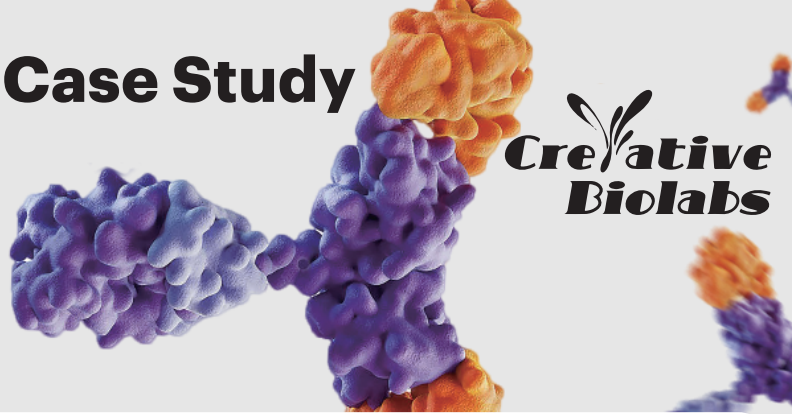


**Creative Biolabs**



| Steps     | Immunogen    | M-F1        |
|-----------|--------------|-------------|
| Primary   | Apr 18, 2016 | F-1(200 µg) |
| Boost-1   | May 09, 2016 | F-1(200 µg) |
| Boost-2   | Jun 2, 2016  | F-1(200 µg) |
| Bleed     | Jul 23, 2016 |             |
| Titrating | Jul 24, 2016 |             |

The diagram illustrates the cloning strategy. It begins with cDNA fragments (VH-1st, VK-1st, VH-2nd, VK-2nd) being amplified by PCR. These fragments are then assembled into a Fab gene construct. The Fab gene is then transformed into T67 cells. The resulting cells are then transformed into a phagemid vector, which is then cut and ligated to form a phagemid.

1 -SLESRGRLVPGTPTLTCTASDINSGMYRQAPKGLGTYGIVETVYAVY  
2 -SLESRGRLVPGTPTLTCTASDINSGMYRQAPKGLGTYGIVETVYAVY  
3 -SLESRGRLVPGTPTLTCTASDINSGMYRQAPKGLGTYGIVETVYAVY  
4 QEDLESRGRLVPGSLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
5 QEDLESRGRLVPGSLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
6 QEDLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
7 -SLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
8 -SLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
9 QEDLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
10 -SLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
11 -SLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY  
12 QEDLESRGRLVPGTPTLTCTSGFSSAAGMYRQAPKGLGTYGIVETVYAVY

| Round              | Conditions                                                                                                                                                       | Input                 | Output               | Enriching factor     |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|----------------------|----------------------|
| 1 <sup>st</sup>    | Target protein: 50 µg/ml F1<br>Washing: 0.1 % Tween20 PBST, 9 times<br>Elution: TEA/basic elution<br>Pre counter select: 2%M-PBS                                 | 2.10×10 <sup>11</sup> | 7.32×10 <sup>4</sup> | 2.87×10 <sup>6</sup> |
| 2 <sup>nd</sup> P  | Target protein: 30 µg/ml F1<br>Washing: 0.2 % Tween20 PBST, 9 times<br>Elution: TEA/basic elution<br>Pre counter select: 2% M -PBS                               | 2.52×10 <sup>11</sup> | 9.24×10 <sup>3</sup> | 2.73×10 <sup>5</sup> |
| 2 <sup>nd</sup> N  | Target protein: no coating<br>Washing: 0.2% Tween20 PBST, 9 times<br>Elution: TEA/basic elution<br>Pre counter select: 2% M -PBS                                 | 3.15×10 <sup>10</sup> | 1.06×10 <sup>3</sup> | 2.97×10 <sup>6</sup> |
| 3 <sup>rd</sup> P  | Target protein: 20 µg/ml F1<br>Washing: 0.3 % Tween20 PBST, 9 times<br>Elution: 50µg/ml mut-F1 22aa peptide competition elution<br>Pre counter select: 2% M -PBS | 2.60×10 <sup>11</sup> | 1.34×10 <sup>7</sup> | 1.94×10 <sup>4</sup> |
| 3 <sup>rd</sup> -N | Target protein: no coating<br>Washing: 0.3% Tween20 PBST, 9 times<br>Elution: 50µg/ml mut-F1 22aa peptide competition elution<br>Pre counter select: 2% M -PBS   | 3.25×10 <sup>10</sup> | 1.47×10 <sup>3</sup> | 2.21×10 <sup>7</sup> |

Phage ELISA

| Phage  | F1 coating (OD450) | No coating (OD450) |
|--------|--------------------|--------------------|
| 1      | ~1.8               | ~0.3               |
| 2      | ~1.6               | ~0.2               |
| 3      | ~1.4               | ~0.2               |
| 4      | ~1.4               | ~0.2               |
| 5      | ~1.2               | ~0.2               |
| 6      | ~1.4               | ~0.2               |
| 7      | ~1.2               | ~0.2               |
| 8      | ~1.5               | ~0.2               |
| 9      | ~1.5               | ~0.2               |
| 10     | ~0.8               | ~0.2               |
| 11     | ~0.8               | ~2.1               |
| 12     | ~0.4               | ~0.2               |
| 13     | ~1.2               | ~0.2               |
| 14     | ~0.8               | ~0.2               |
| 15     | ~0.5               | ~0.2               |
| 16     | ~1.6               | ~0.2               |
| 17     | ~1.4               | ~0.2               |
| 18     | ~0.8               | ~0.2               |
| 19     | ~0.4               | ~0.2               |
| 20     | ~1.6               | ~0.2               |
| 21     | ~1.4               | ~0.2               |
| 22     | ~0.8               | ~0.2               |
| Mexico | ~0.4               | ~0.2               |
| Hades  | ~0.2               | ~0.2               |

**Soluble Monoclonal Antibody ELISA**

OD450

Legend: F1 Coating (dark blue), No coating (light blue)

Labels: Tg1 [lysate], TB

| Sample       | F1 Coating (OD450) | No coating (OD450) |
|--------------|--------------------|--------------------|
| 1            | 1.05               | 0.25               |
| 2            | 1.05               | 0.15               |
| 3            | 0.55               | 0.15               |
| 4            | 0.85               | 0.15               |
| 5            | 0.65               | 0.20               |
| 6            | 0.85               | 0.15               |
| 7            | 0.65               | 0.15               |
| 8            | 0.55               | 0.15               |
| 9            | 0.15               | 0.15               |
| 10           | 0.15               | 0.15               |
| 11           | 0.85               | 0.15               |
| 12           | 0.25               | 0.15               |
| 13           | 0.65               | 0.15               |
| 14           | 0.15               | 0.15               |
| 15           | 0.15               | 0.15               |
| 16           | 1.35               | 0.15               |
| 17           | 0.65               | 0.15               |
| 18           | 0.55               | 0.25               |
| 19           | 0.85               | 0.15               |
| 20           | 0.85               | 0.45               |
| 21           | 0.85               | 0.15               |
| 22           | 0.55               | 0.15               |
| Tg1 [lysate] | 0.15               | 0.15               |
| TB           | 0.15               | 0.15               |

© 2022 Creative Biolabs. | [Contact Us](#)