

## Antibody Sequencing and Recombinant Antibody Production Service

### Risk-free Guaranteed Packages Available

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Antibody Sequencing Packages	Service Contents	Deliverables	Guarantee /EDT /US\$
<p><b>Package #1: Antibody sequencing + Recombinant antibody production + ELISA detection</b></p> <p><b>Antibody type:</b> IgG  <b>Species:</b> Human, Mouse, Rat</p> <p><b>Customer to supply:</b>            1) 200ug antibody, 90% purity            2) 50ug antigen, 90% purity</p>	<p>1) Phase I: Antibody pre-experiment and sequence analysis            2) Phase II: Recombinant antibody production ELISA identification.            3) Phase III: Binding test by ELISA detection</p>	<p><b>First set of deliverables:</b>            COA of ELISA testing result.            Recombinant antibody (<math>\geq 1.0\text{mg}</math>, 90%)</p> <p><b>Second set of deliverables:</b>            COA of antibody sequence.            COA of recombinant antibody synthesis.            COA of peptide coverage map.            4ug plasmid containing the gene insert</p>	<p><b>Guarantee:</b>  <b>Risk-free. Free of charge if the project fails*</b>  <b>Estimated Time:</b> 8-10 weeks  <b>Cost:</b> From \$16,200</p> <p>*We guarantee the same titer of recombinant antibody and the template antibody detected by ELISA in the same order of magnitude.</p> <p>*If the project failure is due to antibody sample impurities (mixed with other antibodies, peptides, or proteins) or interfering agent a report will be provided, and a non-refundable charge of \$3600.</p>
<p><b>Package #2: Antibody Sequencing Only</b></p> <p><b>Antibody type:</b> IgG  <b>Species:</b> Human, Mouse, Rat</p> <p><b>Customer to supply:</b> 200ug antibody, 90% purity</p>	<p>1) Antibody pre-experiment and sequence analysis</p>	<p>COA of antibody sequence.            COA of peptide coverage map.</p>	<p><b>Guarantee:</b>  <b>Risk-free. Free of charge if the project fails*</b>  <b>Estimated Time:</b> 2-4 weeks  <b>Cost:</b> From \$13,500</p> <p>*If the project failure is due to antibody sample impurities (mixed with other antibodies, peptides, or proteins) or interfering agent a report will be provided, and a non-refundable charge of \$3600.</p>
<p><b>Package #3: Antibody sequencing + Recombinant antibody production + ELISA detection</b></p> <p><b>Antibody type:</b> IgG  <b>Species:</b> All species except for human, mouse, and rat</p> <p><b>Customer to supply:</b>            1) 200ug antibody, 90% purity            2) 50ug antigen, 90% purity</p>	<p>1) Phase I: Antibody pre-experiment and sequence analysis            2) Phase II: Recombinant antibody production ELISA identification.            3) Phase III: Binding test by ELISA detection</p>	<p><b>First set of deliverables:</b>            COA of ELISA testing result.            Recombinant antibody (<math>\geq 1.0\text{mg}</math>, 90%)</p> <p><b>Second set of deliverables:</b>            COA of antibody sequence.            COA of recombinant antibody synthesis.            COA of peptide coverage map.            4ug plasmid containing the gene insert</p>	<p><b>Guarantee:</b>  <b>Non-Risk-free. There is a non-refundable charge of \$5400 if the project fails*.</b>  <b>Estimated Time:</b> 10-12 weeks  <b>Cost:</b> From \$18,000</p> <p>*We guarantee the same titer of recombinant antibody and the template antibody detected by ELISA in the same order of magnitude.</p>

<p><b>Package #4: Antibody sequencing only</b></p> <p><b>Antibody type:</b> IgG  <b>Species:</b> All species except for human, mouse, and rat  <b>Customer to supply:</b> 200ug antibody, 90% purity</p>	<p>1) Antibody pre-experiment and sequence analysis</p>	<p>COA of antibody sequence          COA of peptide coverage map</p>	<p><b>Guarantee:</b>  <b>Non-Risk-free. There is a non-refundable charge of \$5400 if the project fails.</b>  <b>Estimated Time:</b> 2-4 weeks  <b>Cost:</b> From \$15,300</p>
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## Antibody Sequencing and Recombinant Protein Production Phases

- ◆ **Phase I:** Antibody pre-experiment and sequence analysis:
  - Determination of the molecular weight of antibodies in reduced, and non-reduced states after glycolysis by LC-MS
  - Detection of impurity protein distribution in antibody samples by SDS-PAGE
  - Determination of amino acid sequence of antibody by ultra-high resolution mass spectrometry
  - Software-aided sequence splicing
  - Accurate antibody sequence generation
  - Analysis of hypervariable region (CDR) and key amino acids
  
- ◆ **Phase II:** Recombinant antibody production
  - Gene synthesis
  - Expression Identification
  - Antibody screening
  - Expression and purification of antibody
  - QC report
  
- ◆ **Phase III:** Binding test by ELISA detection  
 \*See next page

## **Binding Test by ELISA Detection**

(Identification of antibody activity by ELISA)

### **1. Material and Equipment**

Reagent 1: 10mM PBS PH7.4.

Reagent 2: 5% skimmed milk. Add 5g skimmed milk powder into 100ml of reagent 1. Stir until it is completely dissolved.

96-well micro plate, Micropipettor, 37 °C incubator, ELISA analyzer.

### **2. Operation Method**

#### **2.1 Coating antigen:**

- ① Dilute the antigen to 2ug/ml with reagent 1, then add it into a 96-well microtiter plate (100ul/well). Set three wells for each test and placed them at 2-8 °C overnight.

#### **2.2 Blocking ELISA plate:**

- ① Clean the coated enzyme plate 3 times and then dry it. Avoid sample contamination between different detection wells during this process.
- ② Add reagent 2 at 200ul/ well and incubate at 37 °C for 2h.

#### **2.3 Adding recombinant antibody:**

- ① Dilute the antibody: According to the table below, dilute the two groups of antibodies (control antibody and recombinant antibody) with reagent 1 to the working concentration.

The working concentrations are 2, 1, 0.5, 0.25, 0.125, 0.0625, and 0.03125ug/ml.

Name of the sample	Concentration
Control	--mg/ml
Recombinant Antibody	--mg/ml

- ② After diluting, clean the sealing solution of the ELISA plate 3 times, and then dry it.
- ③ Sample incubation: 100ul/well.  
Negative control well: Add reagent 1, 100ul/well.  
Incubate at 37 °C for 30 min.
- ④ Washing: After incubation, put the ELISA plate into the plate washer machine 3 times, and pat the residual liquid on the plate dry.

#### **2.4 Adding the secondary antibody**

- ① The secondary antibody will be diluted with reagent 1 in the ratio of 1: 15000.
- ② Sample incubation: Add 100ul/well to the corresponding well and incubate it at 37 °C for 30min.

③ Washing: For 3 times, and then dry it.

### 2.5 Colour reaction:

Preparation of chromogenic solution: Take out solution A and B from 4 °C in advance and restore to room temperature, then mix them in equal volumes and place them away from light. Add the mixed colour developing solution into the microplate (50ul/well). After slight shaking, the samples will be incubated at 37 °C for 10 min.

### 2.6 Termination and reading:

Add the termination solution, 50ul/well into the microplate, and then read the value in the microplate reader.

Detection wavelength setting: The detection wavelength is 450nm and the reference wavelength is 630nm. The detection results will be exported to Excel.

## 3. Result Analysis

### 3.1 Sample layout in plate:

2	2	2	2	2	2
1	1	1	1	1	1
0.5	0.5	0.5	0.5	0.5	0.5
0.25	0.25	0.25	0.25	0.25	0.25
0.125	0.125	0.125	0.125	0.125	0.125
0.0625	0.0625	0.0625	0.0625	0.0625	0.0625
0.03125	0.03125	0.03125	0.03125	0.03125	0.03125
Negative	Negative	Negative	Negative	Negative	Negative

Control

Recombinant antibody

### 3.2 Result