

# Primacu™ ELISA

## Human Alpha-fetoprotein ELISA Kit

### User instruction

Cat. No. BPE245

Standard Curve Range: 15.63-1000 pg/mL

Sensitivity: 5.21 pg/mL

Size: 5\*96T, 96T, 15\*96T

**\*This product is for research use only, not for use in diagnosis procedures. It's highly recommended to read this instruction entirely before use.**

### Precision

**Intra-Assay Precision** (Precision within an assay) Three samples of known concentration were tested on one plate to assess intra-assay precision.

**Inter-Assay Precision** (Precision between assays) Three samples of known concentration were tested in separate assays to assess inter-assay precision.  
 $CV(\%) = SD/mean \times 100$

Intra/Inter-Assay Precision	Sample	n	Mean	Standard Deviation	CV%
Intra-assay	1	20	31.1	1.7	5.5
Intra-assay	2	20	148	3.7	2.5
Intra-assay	3	20	511	12.2	2.4
Inter-assay	1	20	31.5	3.2	10.1
Inter-assay	2	20	146	14.5	9.9
Inter-assay	3	20	512	47.4	9.2

### Intended Use

This sandwich kit is for the accurate quantitative detection of Human Alpha-fetoprotein (also known as Alpha-fetoprotein ) in serum, plasma, cell culture supernatant. The other biological fluids need to be validated by the end user due to the complexity of natural targets and unpredictable interference.

### Assay Principle

This is an Enzyme-Linked Immunosorbent Assay (ELISA) kit designed to detect Human Alpha-fetoprotein. The assay plate has been pre-coated with mouse anti-Human Alpha-fetoprotein monoclonal antibody. When the sample containing Alpha-fetoprotein is added to the plate, it binds to the antibodies coated on the wells. Then, a horseradish peroxidase conjugated mouse anti-Human Alpha-fetoprotein Antibody is added to the wells and binds to Alpha-fetoprotein in the sample. After washing the wells, substrate solutions are added, and the color intensity is directly proportional to the amount of Human Alpha-fetoprotein present. The reaction is stopped by adding an acidic stop solution, and the absorbance is measured at 450 nm.

### Reagents Provided

Components	Specifications	Storage condition
Pre-coated ELISA Plate	12 × 8 well strips × 1	Return unused wells to the foil pouch containing the desiccant pack and store for up to 1 month at 2-8°C .
Standard Solution	0.2mL × 1 vial	Store at -20°C in a manual defrost freezer until expiration date. Avoid repeated freeze-thaw cycles. Reconstituted working solution should be freshly prepared and discard after use.
Dilution Buffer	20mL × 1 vial	Unopened reagents can store at 2-8°C until expiration date. Store opened reagents at 2-8°C and use within one month.
Stop Solution	6mL × 1 vial	
Substrate Solution A	12mL × 1 vial	
Substrate Solution B	12mL × 1 vial	
Wash Buffer Concentrate (25x)	20mL × 1 vial	
Detection Antibody Concentrate	0.6mL × 1 vial	
User manual	1	
Plate Sealers	2pcs	
Zipper bag	1pcs	

### Material Required But Not Supplied

- Absorbent paper
- Precision pipettes and disposable pipette tips
- Clean tubes
- Deionized or distilled water
- Microplate reader with 450 ± 10nm wavelength filter

### Precautions

- This instruction must be strictly followed in the experiment.
- Store kit at 2 - 8°C. Do not use reagents after expiration date.
- All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

- Substrate Solution B is sensitive to light, don't expose Substrate Solution B to light.
- Stop solution contains acid. Please wear eye, hand and skin protection when using this material.
- The kit should not be used beyond the expiration date.

#### Specimen Collection

**Serum** Allow serum to clot for 30 minutes at room temperature. Then centrifuge at  $1,000 \times g$  for 15 minutes. Collect the supernatant without sediment and assay immediately.

**Plasma** Collect plasma using EDTA or heparin as an anticoagulant. After mix 10-20 minutes, centrifuge samples for 15 minutes at  $1,000 \times g$ . Collect the supernatant without sediment and assay immediately.

**Cell culture supernatant** Remove particulates by centrifugation at  $500 \times g$  for 5 minutes. Collect the supernatant without sediment and assay immediately.

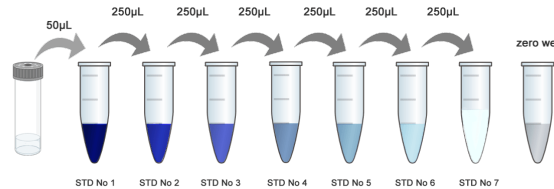
#### Note

- Aliquot and store samples at  $\leq -20^{\circ}\text{C}$ . Avoid repeated freeze-thaw cycles.
- Samples should be brought to room temperature before starting the assay.
- Collect the supernatants carefully. When sediments occurred during storage, centrifugation should be performed again.
- Hemolysis can greatly impact the validity of test results. Take care to minimize hemolysis.

#### Reagent Preparation

- All reagents should be brought to room temperature ( $22 - 28^{\circ}\text{C}$ ) equilibration (at least 30 minutes) before use.
- **Standard Solution** Reconstitute  $50\mu\text{L}$  of the standard solution ( $10 \text{ ng/mL}$ ) with  $450\mu\text{L}$  of Dilution Buffer to generate a stock standard solution of  $1 \text{ ng/mL}$  (Standard No.7). Allow the standard to sit for 15 minutes with gentle agitation prior to making dilutions. Prepare duplicate standard points by serially diluting the standard stock solution ( $1 \text{ ng/mL}$ ) 1:2 with Dilution Buffer to produce  $0.5 \text{ ng/mL}$ ,  $0.25 \text{ ng/mL}$ ,  $0.125 \text{ ng/mL}$ ,  $0.0625 \text{ ng/mL}$ ,  $0.03125 \text{ ng/mL}$  and  $0.015625 \text{ ng/mL}$  solutions. Dilution Buffer serves as the zero standard ( $0 \text{ pg/mL}$ ). Any remaining standard solution should be frozen at  $-20^{\circ}\text{C}$  and used for up to one month. Dilution of standard solutions suggested are as follows:

Concentration	Standard No.	Dilution Steps
$1 \text{ ng/mL}$	Standard No.1	$50\mu\text{L}$ Original Standard + $450\mu\text{L}$ Dilution Buffer
$0.5 \text{ ng/mL}$	Standard No.2	$250\mu\text{L}$ Standard No.7 + $250\mu\text{L}$ Dilution Buffer
$0.25 \text{ ng/mL}$	Standard No.3	$250\mu\text{L}$ Standard No.6 + $250\mu\text{L}$ Dilution Buffer
$0.125 \text{ ng/mL}$	Standard No.4	$250\mu\text{L}$ Standard No.5 + $250\mu\text{L}$ Dilution Buffer
$0.0625 \text{ ng/mL}$	Standard No.5	$250\mu\text{L}$ Standard No.4 + $250\mu\text{L}$ Dilution Buffer
$0.03125 \text{ ng/mL}$	Standard No.6	$250\mu\text{L}$ Standard No.3 + $250\mu\text{L}$ Dilution Buffer
$0.015625 \text{ ng/mL}$	Standard No.7	$250\mu\text{L}$ Standard No.2 + $250\mu\text{L}$ Dilution Buffer
$0 \text{ ng/mL}$	Standard No.0	$250\mu\text{L}$ Dilution Buffer



- **Wash Buffer** Add  $20 \mu\text{L}$  of Wash Buffer Concentrate to  $480 \mu\text{L}$  of deionized or distilled water to prepare  $500 \mu\text{L}$  of  $1 \times$  Wash Buffer. If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved.
- **Solution Substrate** Solution A and B should be mixed together in equal volumes within 10 minutes of use. Protect from light. Each well requires  $200 \mu\text{L}$  of the resultant mixture.
- **Detection Antibody Solution** Add  $0.6 \text{ mL}$  of Detection Antibody Concentrate to  $11.4 \text{ mL}$  Dilution Buffer to prepare  $12 \text{ mL}$  Detection Antibody Solution.

#### Assay Procedure

1. Prepare all reagents, standard solutions, samples and Detection Antibody Solutions as instructed. Bring all reagents to room temperature before use. The assay is performed at room temperature. It is recommended that all standards and samples be assayed in duplicate. Determine the number of strips needed for the assay and insert them into the frames provided. Store unused strips at  $2-8^{\circ}\text{C}$ .
2. Add  $100 \mu\text{L}$  standard or sample per well. Cover the plate with a sealer and incubate 2 hours at room temperature.
3. Discard the contents of the plate. Add  $300 \mu\text{L}$  of  $1 \times$  Wash Buffer to each well and soak for 1 minute. Then aspirate or decant the liquid and pat the wells dry with clean paper towels. Repeat the process twice for a total of three washes. Ensure complete removal of liquid at each step. Note: Improper washes may lead to falsely elevated signals and poor reproducibility.
4. Add  $100 \mu\text{L}$  of the Detection Antibody Solution to each well. Seal the plate and incubate 1 hour at room temperature.
5. Repeat the aspiration/wash as in Step 3.
6. Add  $200 \mu\text{L}$  of Substrate Solution (Pre-mixed in reagent preparation step) to each well. Incubate for 20 minutes at room temperature. Protect from light.
7. Add  $50 \mu\text{L}$  Stop Solution to each well to stop the reaction. The solution color in the wells should change from blue to yellow. Note: If color change does not appear uniform, gently tap the plate to ensure thorough mixing.
8. Determine the optical density (OD value) of each well immediately using a microplate reader set to  $450 \text{ nm}$  within 10 minutes after adding the stop solution.

#### Summary

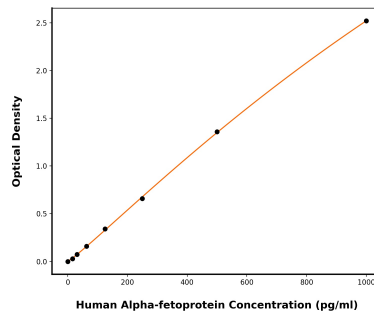
1. Prepare all reagents, standard solutions and samples.
2. Add standard or samples into each well. Incubate for 2 hours at room temperature.
3. Wash the plate 3 times.
4. Add Detection Antibody Solution into each well. Incubate for 1 hour at room temperature.
5. Wash the plate 3 times.
6. Add Substrate Solution. Incubate for 20 minutes at room temperature. Protect from light.
7. Add Stop Solution and color develops.
8. Read the OD value within 10 minutes.

#### Calculation of Result

1. If samples generate values higher than the highest standard, dilute the samples and repeat the assay, then the concentration read from the standard curve must be multiplied by the dilution factor.
2. Calculate the mean absorbance for each standard and sample, and subtract average zero standard optical density.
3. The data been calculated by 4-parameter logistics curve-fitting algorithm.

#### Typical Data

This standard curve is for demonstration purpose only. A standard curve should be generated with each assay.



Concentration	OD Value	Average	Corrected
1000 pg/ml	2.483	2.6	2.519
	2.717		
500 pg/ml	1.371	1.438	1.357
	1.505		
250 pg/ml	0.748	0.74	0.659
	0.732		
125 pg/ml	0.443	0.423	0.342
	0.403		
62.5 pg/ml	0.23	0.241	0.16
	0.252		
31.25 pg/ml	0.164	0.155	0.074
	0.147		
15.625 pg/ml	0.111	0.108	0.027
	0.105		
0 pg/ml	0.076	0.081	0
	0.086		

#### Troubleshooting

Possible Case	Solution
<b>High Background</b>	
<ul style="list-style-type: none"> <li>Insufficient washes</li> </ul>	<ul style="list-style-type: none"> <li>See washing procedure on the user manual</li> <li>Increase number of washes</li> </ul>
<b>Weak Signal</b>	
<ul style="list-style-type: none"> <li>Improper washing</li> <li>Incorrect incubation temperature</li> <li>Reagent are contaminated</li> <li>Pipette are not clean</li> </ul>	<ul style="list-style-type: none"> <li>Increasing duration of soaking steps</li> <li>incubate at room temperature</li> <li>Use new one</li> <li>Pipette should be clean</li> </ul>
<b>No Signal</b>	
<ul style="list-style-type: none"> <li>Reagents added in incorrect order, or incorrectly prepared</li> <li>Standard curve looks fine but there is no signal in the sample wells</li> <li>Standard wells no signal but there is a signal in the sample wells</li> </ul>	<ul style="list-style-type: none"> <li>Review manual and repeat assay</li> <li>Check if the samples were handled and stored correctly.</li> <li>Check that standard was handled according to manual.</li> </ul>
<b>Poor Standard Curve</b>	
<ul style="list-style-type: none"> <li>Plate not developed long enough</li> <li>Incorrect procedure</li> <li>Imprecise / inaccurate pipetting</li> </ul>	<ul style="list-style-type: none"> <li>Increase Substrate Solution incubation time</li> <li>Review manual and repeat the assay to establish the standard curve.</li> <li>Check and calibrate the pipettes</li> </ul>

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