

## Human HGFAC ELISA Kit

Cat #: orb1808993 (manual)

Size: 96 tests

This kit is used to quantify the amount of HGFAC in samples such as human serum, plasma, tissue homogenate and other biological fluids. Please read the manual carefully and check the reagent components before use. If you have any questions, please contact biorbyt, we will provide you with technical support.

For research use only, not for clinical diagnosis.

### Detection Principle

This kit is based on sandwich enzyme-linked immune-sorbent assay technology. Purified HGFAC antibody was coated in the microplates, then the standard or the samples to be tested are added to the coated plate wells. After the antigen fully reacts with the coated antibody, the biotinylated HGFAC antibody and streptavidin labelled with HRP are added in turn. The biotin and streptavidin form high-intensity non-covalent binding. After sufficient washing, the substrate TMB is added for colour development. TMB is converted to blue under the catalysis of HRP enzyme and finally to yellow under the action of stop solution. The colour shading is proportional to the HGFAC content in the sample. Finally, the OD value was determined by microplate reader at the wavelength of 450 nm, and the concentration of HGFAC in the sample was calculated by plotting the standard curve.

### Product Composition

| Reagents                           | Specifications (96T) | Storage Conditions |
|------------------------------------|----------------------|--------------------|
| Antibody-Coated Slats              | 8×12                 | -20°C              |
| Standard                           | 2 tubes              | -20°C              |
| S1 Standard/Sample Dilution Buffer | 45 ml×1 bottle       | -20°C              |
| Detection Solution A               | 120µl×1 tube         | -20°C              |
| Detection Solution B               | 120µl×1 tube         | -20°C              |
| Washing Buffer (Concentrated, 30×) | 20ml×1 bottle        | 2-8°C              |
| TMB Substrate (Avoid direct light) | 9ml×1 bottle         | 2-8°C              |
| Stop Solution                      | 6ml×1 bottle         | 2-8°C              |
| Plate Sealer                       | 4 pieces             |                    |
| Manual                             | 1 copy               |                    |

### Required Instruments and Reagents

1. Microplate reader (wavelength: 450nm)
2. Precision single (0.5-10 $\mu$ L, 2-20 $\mu$ L, 20-200 $\mu$ L, 200-1000 $\mu$ L) and multi-channel pipette with disposable tips (calibration is required before use.)
3. Automated plate washer or wash bottles
4. 37°C incubator
5. Deionized or distilled water
6. Coordinate paper
7. Measuring cylinder

### Precautions

1. The kit is stored in 4°C&-20°C, the dissolved but unused standard is recommended for disposal. Do not mix kit components from different sources or batch numbers, use this product within the expiration date.
2. When the concentrated washing solution is taken out at low temperature, there may be crystal precipitation, and it can be dissolved by heating in the water bath before dilution, which does not affect the use.
3. Pipettes should be calibrated before and used for each step and to avoid errors. It is recommended to control the sampling time within 5 minutes. If there are a large number of samples, it is recommended to use a multi-channel pipette for sampling.
4. Please make a standard curve at the same time of each determination, and it is better to make a double-check well. If the content of the substance to be tested in the sample is higher than the upper limit of the reagent kit (the OD value of the sample is greater than the OD value of the first well of the standard well), please dilute a certain multiple with the sample dilution buffer before determination. The total dilution multiple shall be multiplied during calculation.
5. In order to avoid cross contamination, remember to replace the tip when adding different concentrations of standard, different samples and different reagents. The plate sealer is only for single use.
6. The TMB substrate should be kept colorless before adding. Do not use the TMB substrate when it turned blue.
7. Strictly follow the manual, and the test results must be based on the microplate reader reading.

### Sample Collection and Storage

1. **Serum:** Blood coagulated naturally at room temperature for 60~120 min and centrifuged for 20 min (1000g). Collect supernatant carefully. If precipitation occurs during storage, centrifuge again to avoid repeated freezing and thawing.
2. **Plasma:** Select EDTA or citric acid as anticoagulant according to sample requirements and centrifuge for about 20 min (1000g). Collect the supernatant carefully and centrifuge again if precipitation occurs during storage.

3. **Other biological fluids:** When testing secreted components, collect using sterile tubes, centrifuge at 1000g for about 20 minutes, and collect the supernatant.

#### 4. Tissue homogenate:

1) Take an appropriate amount of tissue blocks, wash with pre-chilled PBS to remove blood, weigh, and set aside (if the tissue blocks are large, cut them into smaller pieces before homogenizing).

2) Multiple homogenization methods can be used simultaneously to achieve better disruption: first, transfer the tissue blocks into a glass homogenizer, add 5-10 mL of pre-chilled PBS for thorough grinding, and perform this process on ice; the resulting homogenate can be further processed using ultrasonic disruption or repeated freeze-thaw cycles.

3) Centrifuge the prepared homogenate at 5000×g for 5 minutes and collect the supernatant.

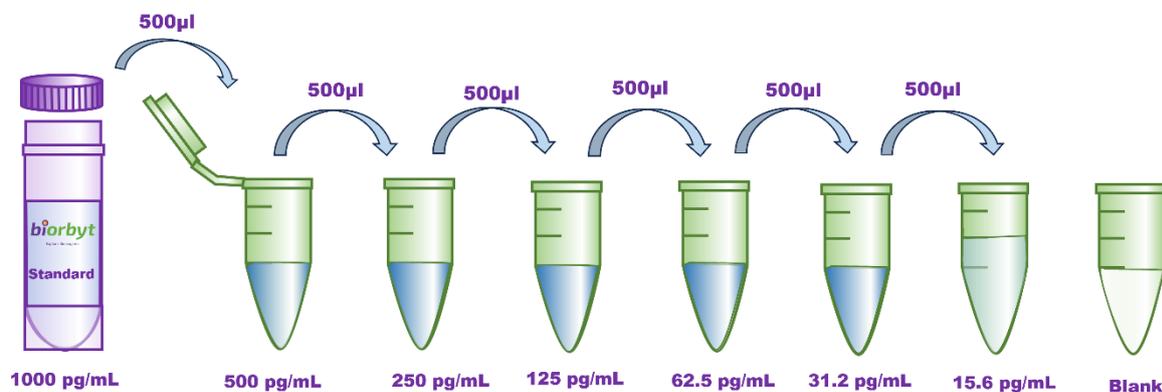
5. If the sample cannot be tested immediately, dispense it according to the minimum amount of use, and store it in -20°C to -70°C to avoid repeated freezing and thawing. Avoid haemolytic or hyperlipidaemia samples.

### Reagent Preparation

1. Reagent reheating: Please reheat the reagent kit and the sample to be tested at room temperature within 30 minutes before the test.

2. Preparation of Washing Buffer: Dilute the concentrated Washing Buffer (30×) to Washing Buffer working solution (1×) with double distilled water or deionized water and keep it as standby.

3. Gradient dilution of standard: Take 1 ml of Standard/Sample Dilution Buffer (S1) into the lyophilized standard, allow it to stand for 15 min until it is completely dissolved, then gently mix it with a concentration of 1000pg/ml, take 6 EP tubes, add 500ul Standard/Sample Dilution Buffer (S1) each EP tube, and dilute twice according to the following concentration: 500, 250, 125, 62.5, 31.2, 15.6pg/ml were diluted. 1000pg/ml is the highest concentration of the standard curve, and the Standard/Sample Dilution Buffer (S1) is the zero point (0pg/ml) of the standard curve. The Standard Stock Solutions (1000pg/ml) that has not been used up should be discarded.



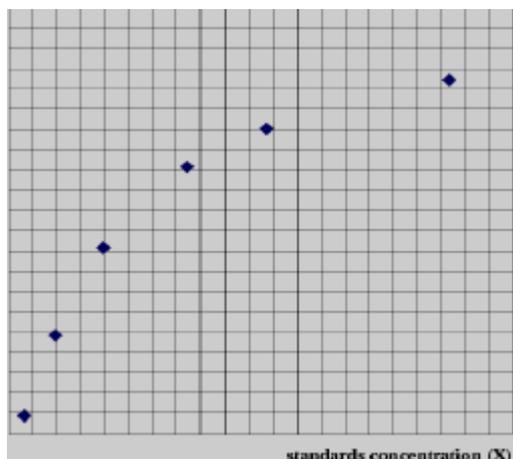
4. Detection Solution A and Detection Solution B: Before use, gently shake by hand a few times or briefly centrifuge to bring any liquid deposited on the tube walls or bottle cap down to the bottom of the tube. Just before use, dilute each solution 1:100 with the diluent (e.g., 10  $\mu$ L of Detection Solution A in 990  $\mu$ L of diluent) and mix thoroughly. Prepare the total amount needed for each experiment (100  $\mu$ L per well) based on prior calculations, and prepare an extra 0.1–0.2 mL to ensure sufficient volume.

### Operation Steps

1. Sample addition: According to the required amount for the experiment, take out the corresponding antibody-coated plates, and add 100  $\mu$ l per well of the prepared standards, standard zero point, and samples to be tested at the bottom of the wells.
2. Incubation: Seal the plate with sealing tape and incubate at 37°C for 120 minutes.
3. Discard the liquid, flick dry, no washing required.
4. Add Detection Solution A working solution (prepared freshly before use): add 100  $\mu$ l per well.
5. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.
6. Washing: Carefully remove the sealing tape, discard the liquid, flick dry, fill each well with 350  $\mu$ l washing solution, let stand for 1-2 minutes, then discard; repeat this process 3 times, and finally blot dry on absorbent paper.
7. Add Detection Solution B working solution (prepared freshly before use): add 100  $\mu$ l per well.
8. Incubation: Seal the plate with sealing tape and incubate at 37°C for 60 minutes.
9. Washing: Follow the same washing procedure as described above (step 6), wash the plate 5 times.
10. Color development: Add 90  $\mu$ l of substrate solution to each well, seal the plate, and incubate at 37°C for 15-25 minutes.
11. Termination: Add 50  $\mu$ l of stop solution to each well (the color changes from blue to yellow).
12. Measurement: Use a microplate reader to measure the absorbance (OD value) at 450 nm for each well. The measurement should be performed within 5 minutes after adding the stop solution.

### Result Judgment

1. The OD value of each standard and sample minus the OD value of the blank well is the final value, and if a duplicate well is made, its mean value shall be calculated.
2. Take the absorbance OD value as the ordinate (Y) and the corresponding standard concentration as the abscissa (X) to generate the corresponding standard curve. The HGFAC content of the sample can be calculated from the standard curve according to its OD value. If the OD value of the sample is higher than the upper limit of the standard curve, perform the appropriate dilution during the test, and then multiply the concentration of the sample by the corresponding dilution.



This drawing is for reference only and shall be based on the standard curve drawn in the actual test

### Kit Performance

The difference between batches should be less than 10%

### Detection Range

15.6 pg/ml -1000 pg/ml

### Sensitivity

6.4 pg/ml

### Troubleshooting

| Problems  | Possible Causes  | Solutions   |
|-----------|--|---|
| No signal | Mixing Reagents with Different ELISA kits or Batch Numbers | Recheck the label of the reagents to make sure that all components are in the testing kit being used. Do not mix reagents of different testing kits or batch numbers. |
|           | Missing antibody, enzyme and chromogenic agent             | Check the operation procedure and be careful not to omit adding.  |
|           | HRP enzyme contaminated with sodium azide                  | Re-preparation of reagent, no sodium azide  |
|           | Wrong reagent preparation/use                              | Redo the test, operate in strict accordance with the manual, and see the labels clearly before  |

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|  |  | each preparation and use   |  |
| Weak signal  | Reagents Expires expiration date                           | Check product validity   |  |
|  | Insufficient incubation time                               | Check the incubation time  |  |
|  | Use of contaminated reagents                               | Check if reagent is contaminated   |  |
|  | Incorrect instrument setting, filter mismatch              | Whether the instrument is set correctly and the filter is used, etc.   |  |
|  | Washing operation is not standard                          | If the washing is insufficient, increase the number of washing times or extend the washing time  |  |
|  |  | Wash the bottle, each well shall be completely filled with washing buffer, and pour out quickly  |  |
|  |  | If a plate washer is used, it shall be calibrated and set to a volume sufficient to fill each hole and the inside of the plate shall not touch the equipment |  |
| Check whether there is residual washing liquid in each well or the volume of sample added in each well is accurate |  |  |  |
| You can add a 30 second soak between washings  |  |  |  |
| High background  | Improper incubation temperature and time in the experiment | Determine the appropriate incubation temperature and time for each test step   |  |
|  | Excessive enzyme addition                                  | Check whether the regulating amount of pipette is correct before adding enzyme   |  |
|  |  | Check dilution and perform titer determination if necessary  |  |
| The standard curve is good, but the sample wells have no signal  | Low content of target in sample or no target in sample     | Set the positive control and repeat the experiment   |  |
|  | Sample matrix effect influence detection                   | Test again after re-diluting sample  |  |

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| <p>The standard curve is good, but the sample wells have high signal</p> | <p>The content of sample to be tested exceeds the standard curve range</p> | <p>Test again after re-diluting sample</p> |
|--|--|--|