Technical Manual

Human Hyaluronic Acid ELISA Kit

- **Catalogue Code: HDES0088**
- **Antibody ELISA Kit**
- **Research Use Only**

1. Test principle

This ELISA kit uses Sandwich-ELISA as the method to detect the Hyaluronic Acid (HA) in human serum. The ELISA Microtiter plate provided in this kit has been pre-coated with HA monoclonal antibody. Samples and HA polyclonal antibody are sequentially added to the ELISA Microtiter plate wells, after wash, add HRP Conjugate. If HA present in the sample, it will combine with the pre-coated HA monoclonal antibody to form antibody-antigen-antibody- HRP Conjugate compound. Wash the Microtiter plate to remove unbound HRP Conjugates. The substrate reagent is added to initiate the color developing reaction. The presence of HA can be determined by measurin0g the absorbance value by using a microplate reader with 450 nm wavelength. The OD value is proportional to the concentration of HA. You can calculate the concentration of HA in the samples by comparing the OD of the samples to the standard curve.

2. Kit components

Item	Specifications
ELISA Microtiter plate	96 wells
Standard Liquid	0.5 mL each (50 ng/mL, 100 ng/mL, 200 ng/mL, 400 ng/mL, 600 ng/mL)
25×Concentrated Wash Buffer	20 mL
HRP Conjugate	5 mL×2
Binding Antibody	5 mL
Substrate Reagent A	5 mL
Substrate Reagent B	5 mL
Stop Solution	5 mL
Plate Sealer	3 pieces
Sealed Bag	1 piece
Manual	1 copy

Note: All reagent bottle caps must be tightened to prevent evaporation and microbial pollution.

3. Other materials required but not supplied

- Microplate Reader with 450 nm wavelength filter or dual-wavelength (450/630 nm)
- High-precision transferpettor, EP tubes and disposable pipette tips
- 37° C Incubator or water bath
- Deionized water
- Absorbent paper

4. Notes

1. Please read the manual carefully before use, changes of operation may result in unreliable results.
2. Wear gloves and work clothes during experiment, and the disinfection and isolation system should be strictly executed. All the waste should be handled as contaminant.
3. The stop solution is corrosive, it should be avoided to contact with skin and clothing. Wash immediately with plenty of water if contact it carelessly.
4. The ELISA Microtiter plate obtained from cold storage conditions should be adjusted to room temperature before use. The unused plate should be kept in a sealed bag with desiccant.
5. Concentrated washing liquid at low temperature condition is easy to crystallization, it should be adjusted to room temperature in order to dissolve completely before use.
6. The results shall depend on the readings of the micro-plate Reader.
7. **Each reagent is optimized for use in the HDES0088. Do not substitute reagents from any other manufacturer into the test kit. Do not combine reagents from other HDES0088 with different lot numbers.**
8. All the samples and waste material should be treated as infective material according to the relevant rules of biosafety.
9. The blood source raw material used in the preparation of the kit has been inactivated at 60°C, all of them were test by HBsAg, anti-HCV, anti-HIV, anti-TP test kits approved by the SFDA, and they present negative result, however there is no test method can completely guarantee absolute safety, furthermore, the sample is derived from human blood. Therefore both samples and kits should be treated as potentially infectious reagents.

5. Storage and expiry date

Store at 2-8° C. Avoid freeze.
Please store the opened plate at 2-8° C, the shelf life of the opened kit is up to 3 month.
Expiry date: expiration date is on the packing box.

6. Sample preparation

1. **Serum:** Human serum can be used as detected sample. Fresh collected serum samples should be fully centrifuged, then take clear liquid for assay. The suspended fibrous protein may cause a false positive result if not fully precipitated. Obviously contaminated samples can't be detected. Samples can be stored at 2-8°C for 24 h and stored at -20°C for 4 weeks. Avoid freeze-thaw cycles. Freezing samples should be mixed fully before test.
   Note: It is suggested to draw blood in the statement of an empty stomach.
2. Avoid of using hemolysis, turbidity, or lipid blood samples

3. **Wash Buffer:** The **25×Concentrated Wash Buffer** should be adjusted to room temperature to make the sediment dissolved fully before use, then dilute it with deionized water at 1:24.

7. Assay procedure

Restore all reagents and samples to room temperature (25° C) before use. All the reagents should be mixed thoroughly by gently swirling before pipetting. Avoid foaming. The unused ELISA Microtiter plate should be sealed as soon as possible and stored at 2-8° C.

1. **Number:** number the sample and standard in order (multiple well), and keep a record of standard wells and sample wells.
2. **Add sample:** add 50 μL of **Standard or Sample** per well, set the blank control well.
3. **Add Binding Antibody:** add 50 μL of HA polyclonal Antibody per well, keep the blank control well empty.
4. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 60 min at 37° C in shading light.
5. **Wash:** remove the plate sealer and aspirate the liquid of each well. Repeat the washing procedure for 5 times with **Wash Buffer** and immerse for 30-60 sec each time. Invert the plate and pat it against thick clean absorbent paper (If bubbles exist in the wells, clean tips can be used to prick them).
6. **Add HRP Conjugate:** add 100 μL of HRP Conjugate per well, keep the blank control well empty.
7. **Incubate:** gently tap the plate to mix thoroughly. Cover the ELISA Microtiter plate with sealer. Incubate for 30 min at 37° C in shading light.
8. **Wash:** repeat step 5.
9. **Add substrate:** add 50 μL of **Substrate Reagent A** and 50 μL of **Substrate Reagent B** to each well. Gently tap the plate to mix thoroughly. Cover with a new plate sealer. Incubate for 15 min at 37° C in shading light.
10. **Stop reaction:** add 50 μL of **Stop Solution** to each well, gently tap the plate to mix thoroughly.
11. **OD Measurement:** set the Microplate Reader wavelength at 450 nm to detect A value of each well, zero set with blank well.

Note: If the A value of highest concentration standard (600 ng/mL) ≥0.8, the test result is valid.

8. Result analysis

1. Create a standard curve by plotting the absorbance of each standard on the y-axis against the concentration on the x-axis to draw a standard curve. Add average absorbance value of sample to standard curve to get corresponding concentration.
2. If the A value of sample is higher than highest concentration standard (600 ng/mL), the sample should be diluted. And the concentration calculated from the standard curve must be multiplied by the dilution factor.

9. Reference value

Normal reference＜120 ng/mL.
The critical value is based on the determination of HA in large number of human serum samples, set X±2SD as the upper limit of the normal value.

10. Product performance

1. **Accuracy:** the recovery rate should be within 85-115%.
2. **Limit of detection:** should not be more than 50 ng/mL.
3. **Linear range:** in the range of [50 ng/mL, 600 ng/mL], the correlation coefficient r≥0.990.
4. **Repeatability:** the test was repeated with high and low concentration levels of samples for 10 times, coefficient of variation (CV) ≤ 15%.
5. **Specificity:** there is no cross-reactivity with laminin LN, Type III procollagen peptide and TypeⅣ collagen at the concentration of 780 ng/mL, 600 ng/mL, 800 ng/mL respectively.

11. Limitations of this test method

1. This product can only be used for the determination of serum samples and can not be used for other body fluid samples.
2. To avoid interfering with the results, serum with high blood lipids and hemolysis should be avoided. Samples with obvious contamination should not be used for testing as they may lead to erroneous experimental results.

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