Human Lambda ELISA Kit

Catalog No.: IRKTAH1138

Lot No.: SAMPLE

The total Human Lambda test kit is a highly sensitive two-site enzyme linked immunoassay (ELISA) for measuring Lambda in Human Biological Samples.

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Assay Principle

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Lambdapresent in samples reacts with the anti-Lambdaantibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-Lambdaantibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound Lambda. Following another washing step, the enzyme bound to the immunosorbent is assayed by the addition of a chromogenic substrate, 3,3',5,5'-tetramethylbenzidine (TMB). The quantity of bound enzyme varies directly with the concentration of Lambda in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Lambda in the test sample. The quantity of Lambda in the test sample can be interpolated from the standard curve constructed from the standards, and corrected for sample dilution.

Reagents Provided

1. DILUENT CONCENTRATE (Running Buffer)

One bottle containing 50 ml of a 5X concentrated diluent running buffer.

2. WASH SOLUTION CONCENTRATE

One bottle containing 50 ml of a 20X concentrated wash solution.

3. ENZYME-ANTIBODY CONJUGATE 100X

One vial containing 150 uL of affinity purified anti Human Lambda antibody conjugated with horseradish peroxidase in a stabilizing buffer.

4. CHROMOGEN-SUBSTRATE SOLUTION

One vial containing 12 mL of 3,3',5,5'-tetramethybenzidine (TMB) and hydrogen peroxide in citric acid buffer at pH 3.3.

5. STOP SOLUTION

One vial containing 12 ml 0.3 M sulfuric acid.

6. ANTI-HUMAN Lambda ELISA MICRO PLATE

Twelve removable eight (8) well micro well strips in well holder frame. Each well is coated with affinity purified anti-Human Lambda.

Storage and Stability

1. DILUENT

The 5X Diluent Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions should be stored at 4-8C.

2. WASH SOLUTION

The 20X Wash Solution Concentrate is stable until the expiration date. The 1X working solution is stable for at least one week from the date of preparation. Both solutions can be stored at room temperature (16-25C) or at 4-8C.

3. ENZYME-ANTIBODY CONJUGATE

Undiluted horseradish peroxidase anti-Lambda conjugate should be stored at 4-8C and diluted immediately prior to use. The working conjugate solution is stable for up to 1 hour when stored in the dark.

4. CHROMOGEN-SUBSTRATE SOLUTION

The Substrate Solution should be stored at 4-8C and is stable until the expiration date.

5. STOP SOLUTION

The Stop Solution should be stored at 4-8□C and is stable until the expiration date.

6. ANTI-HUMAN Lambda ELISA MICRO PLATE

Anti-Human Lambda coated wells are stable until the expiration date, and should be stored at 4-8□C in sealed foil pouch with desiccant pack.

7. HUMAN Lambda CALIBRATOR

Long Term Storage: Upon receipt, aliquot the calibrator and store them frozen. They will be stable until expiration date. Short Term Storage: the calibrator is stable for up to 14 days at 4ºC. The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

Sample Collection

Blood should be collected by venipuncture. The serum should be separated from the cells after clot formation by centrifugation. For plasma samples, blood should be collected into a container with an anticoagulant and then centrifuged. Care should be taken to minimize hemolysis, excessive hemolysis can impact your results. Assay immediately or aliquot and store samples at -20□C. Avoid repeated freezethaw cycles

Reagent Preparation

1. DILUENT CONCENTRATE

The Diluent Solution supplied is a 5X Concentrate and must be diluted 1/5 with distilled or deionized water (1 part buffer concentrate, 4 parts dH2O)

2. WASH SOLUTION CONCENTRATE

The Wash Solution supplied is a 20X Concentrate and must be diluted 1/20 with distilled or deionized water (1 part buffer concentrate, 19 parts dH2O). Crystal formation in the concentrate is not uncommon when storage temperatures are low. Warming of the concentrate to 30-35□C before dilution can dissolve crystals.

3. ENZYME-ANTIBODY CONJUGATE

Calculate the required amount of working conjugate solution for each microtitre plate test strip by adding 10uL Enzyme-Antibody Conjugate to 990uL of 1X Diluent for each test strip to be used for testing. Mix uniformly, but gently. Avoid foaming.

4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

5. STOP SOLUTION

Ready to use as supplied

6. ANTI-HUMAN Lambda ELISA MICRO PLATE

Ready to use as supplied. Unseal Microtiter Pouch and remove plate from pouch. Remove all strips and wells that will not be used in the assay and place back in pouch and re-seal along with desiccant.

Assay Procedure

The assay for quantification of Lambda in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/100,000 is appropriate for most serum/plasma samples. For absolute quantification, samples that yield results outside the range of the standard curve, a lesser or greater dilution might be required. If unsure of sample level, a serial dilution with one or two representative samples before running the entire plate is highly recommended.

1. To prepare a 1/100,000 dilution of sample, transfer 2uL of sample to 1998uL of 1X diluent. This gives you a 1/1000 dilution. Next, dilute the 1/1000 samples by transferring 10uL, to 990uL of 1X diluent. You now have a 1/100,000 dilution of your sample. Mix thoroughly at each stage.

3. Pipette 100µL of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for thirty (30 ± 2) minutes. Keep plate covered and level during incubation.

5. Following incubation, aspirate the contents of the wells.

6. Completely fill each well with appropriately diluted Wash Solution and aspirate. Repeat three times, for a total of four washes. If washing manually: completely fill wells with wash buffer, invert the plate then pour/shake out the contents in a waste container. Follow this by sharply striking the wells on absorbent paper to remove residual buffer. Repeat 3 times for a total of four washes.

7. Pipette 100µL of appropriately diluted Enzyme Antibody Conjugate to each well. Incubate at room temperature for twenty (20 ± 2) minutes. Keep plate covered in the dark and level during incubation.

8. Wash and blot the wells as described in Steps 5/6.

9. Pipette 100µL of TMB Substrate Solution into each well.

10. Incubate in the dark at room temperature for precisely ten (10) minutes.

11. After ten minutes, add 100µL of Stop Solution to each well.

12. Determine the absorbance (450 nm) of the contents of each well. Calibrate the plate reader to manufacturer's specifications.

Standard	Ng/ml	Volume Added	Volume of Diluent
6	200	30 ul of Calibrator	720ul
5	100	300ul #6	300ul
4	50	300ul #5	300ul
3	25	300ul #4	300ul
2	12.5	300ul #3	300ul
1	6.25	300ul #2	300ul
0	0		600ul

Results

1. Subtract the average background value from the test values for each sample.

2. Using the results observed for the standards construct a Standard Curve. The appropriate curve fit is that of a four-parameter logistics curve. A second order polynomial (quadratic) or other curve fits may also be used.

3. Interpolate test sample values from standard curve. Correct for sera dilution factor to arrive at the Lambda concentration in original samples