



EAGLE
BIOSCIENCES

Human Kappa ELISA Assay Kit

Catalog Number: HKE39-K01

96 Wells

For Research Use Only.

v. 2.0 (08.22.22)

Eagle Biosciences, Inc.
20A Northwest Blvd., Suite 112, Nashua, NH 03063
Phone: 617-419-2019 Fax: 617-419-1110
www.EagleBio.com



INTENDED USE

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

ASSAY PRINCIPLE

The principle of the double antibody sandwich ELISA is represented in Figure 1. In this assay the Kappa present in samples reacts with the anti-Kappa antibodies which have been adsorbed to the surface of polystyrene microtitre wells. After the removal of unbound proteins by washing, anti-Kappa antibodies conjugated with horseradish peroxidase (HRP), are added. These enzyme-labeled antibodies form complexes with the previously bound Kappa. 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 Kappa in the sample tested; thus, the absorbance, at 450 nm, is a measure of the concentration of Kappa in the test sample. The quantity of Kappa in the test sample can be interpolated from the standard curve constructed from the standards and corrected for sample dilution.

REAGENTS

| Component | Amount |
|---|-----------------------|
| 5X Diluent Concentrate (Running Buffer) | 50 mL |
| 20X Wash Solution Concentrate | 50 mL |
| 100X Enzyme Antibody Conjugate | 1 vial (100 μ L) |
| Chromogen Substrate Solution | 1 vial (12 mL) |
| Stop Solution | 12 mL |
| Warning: Avoid contact with skin | |
| Streptavidin-HRP conjugate reagent | 12 mL |
| Anti-Human Kappa Microplate | 12 x 8 wells |
| Human Kappa Calibrator | 1 vial |

REAGENT PREPARATION

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).

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.



Enzyme- Antibody Conjugate

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

Chromogen- Substrate Solution

Ready to use as supplied.

Stop Solution

Ready to use as supplied.

Anti-Human Kappa ELISA Microplate

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.

Human Kappa Calibrator

The calibrator is now at a concentration of 9.49 $\mu\text{g}/\text{mL}$. Human Kappa standards need to be prepared immediately prior to use (see chart below). Mix well between each step. Avoid foaming.

| Standard | ng/mL | Volume added to 1X Diluent | Volume of 1X Diluent |
|----------|-------|---|----------------------|
| 6 | 200 | 16 μL Human Kappa Calibrator | 300 μL |
| 5 | 100 | 300 μL standard 6 | 300 μL |
| 4 | 50 | 300 μL standard 5 | 300 μL |
| 3 | 25 | 300 μL standard 4 | 300 μL |
| 2 | 12.5 | 300 μL standard 3 | 300 μL |
| 1 | 6.25 | 300 μL standard 2 | 300 μL |
| 0 | 0 | | 600 μL |

STORAGE AND STABILITY

The expiration date for the package is stated on the box label.



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 4-8°C.

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(16-25°C) or at 4-8°C.

3. Enzyme-Antibody Conjugate

Undiluted horseradish peroxidase anti-Kappa conjugate should be stored at 4-8°C 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 4-8°C 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 Kappa Microplate

Anti-Human Kappa 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 Kappa 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 4°C. The working standard solutions should be prepared immediately prior to use and are stable for up to 8 hours.

Indications of Instability:

If the test is performing correctly, the results observed with the standard solutions should be within 20 % of the expected values.

SPECIMEN COLLECTION AND HANDLING

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 freeze-thaw cycles.



1. Precautions

For any sample that might contain pathogens, care must be taken to prevent contact with open wounds.

2. Additives and Preservatives

No additives or preservatives are necessary to maintain the integrity of the specimen. Avoid azide contamination.

3. Known Interfering Substances

Azide and thimerosal at concentrations higher than 0.1% inhibits the enzyme reaction.

MATERIALS REQUIRED

See "REAGENTS"

MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2 μ L to 200 μ L) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H₂O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

ASSAY PROTOCOL

DILUTION OF SAMPLES

The assay for quantification of Kappa 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 2 μ L of sample to 1998 μ L of 1X diluent. This gives you a 1/1000 dilution. Next, dilute the 1/1000 samples by transferring 10 μ L, to 990 μ L of 1X diluent. You now have a 1/100,000 dilution of your sample. Mix thoroughly at each stage.

PROCEDURE

1. Bring all reagents to room temperature before use.
2. Pipette 100 μ L of
 - a) Standard 0 (0.0 ng/ml) in duplicate
 - b) Standard 1 (6.25 ng/ml) in duplicate
 - c) Standard 2 (12.5 ng/ml) in duplicate



- d) Standard 3 (25 ng/ml) in duplicate
 - e) Standard 4 (50 ng/ml) in duplicate
 - f) Standard 5 (100 ng/ml) in duplicate
 - g) Standard 6 (200 ng/ml) in duplicate
3. Pipette 100 μ L of sample (in duplicate) into predesignated 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 100 μ L 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 100 μ L 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 100 μ L 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.

STABILITY

The absorbance of the final reaction mixture can be measured up to 2 hours after the addition of the Stop Solution. However, good laboratory practice dictates that the measurement be made as soon as possible.

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 Kappa concentration in original samples.

LIMITATIONS

1. Reliable and reproducible results will be obtained when the assay procedure is carried out with a complete understanding of the information contained in the package insert instructions and with adherence to good laboratory practice.
2. Factors that might affect the performance of the assay include proper instrument function, cleanliness of glassware, quality of distilled or deionized water, and accuracy of reagent and sample pipettings, washing technique, incubation time or temperature.
3. Do not mix or substitute reagents with those from other lots or sources.



WARRANTY INFORMATION

Eagle Biosciences, Inc. warrants its Product(s) to operate or perform substantially in conformance with its specifications, as set forth in the accompanying package insert. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Eagle Biosciences within the expiration period of the Product(s) by the Buyer. In addition, Eagle Biosciences has no obligation to replace Product(s) as result of a) Buyer negligence, fault, or misuse, b) improper use, c) improper storage and handling, d) intentional damage, or e) event of force majeure, acts of God, or accident.

Eagle Biosciences makes no warranties, either expressed or implied, except as provided herein, including without limitation thereof, warranties as to marketability, merchantability, fitness for a particular purpose or use, or non-infringement of any intellectual property rights. In no event shall the company be liable for any indirect, incidental, or consequential damages of any nature, or losses or expenses resulting from any defective product or the use of any product. Product(s) may not be resold, modified, or altered for resale without prior written approval from Eagle Biosciences, Inc.

For further information about this kit, its application or the procedures in this kit, please contact the Technical Service Team at Eagle Biosciences, Inc. at info@eaglebio.com or at 866-411-8023.