



## 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 dH<sub>2</sub>O). 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 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.

## 4. CHROMOGEN-SUBSTRATE SOLUTION

Ready to use as supplied.

## 5. STOP SOLUTION

Ready to use as supplied.

## 6. ANTI-MOUSE IgG1 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.

## 7. MOUSE IgG1 CALIBRATOR

The calibrator is now at a concentration of 1.86 mg/ml. **Mouse IgG1 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
A	18600	5 µl Mouse IgG1 Calibrator	495 µl
6	200	10 µl standard A	920 µl
5	100	250 µl standard 6	250 µl
4	50	250 µl standard 5	250 µl
3	25	250 µl standard 4	250 µl
2	12.5	250 µl standard 3	250 µl
1	6.25	250 µl standard 2	250 µl
0	0		500 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-IgG1 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-MOUSE IgG1 ELISA MICRO PLATE

Anti-Mouse IgG1 coated wells are stable until the expiration date, and should be stored at 4-8°C in sealed foil pouch with desiccant pack.

## 7. MOUSE IgG1 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.

## MATERIAL PROVIDED

See "REAGENTS"

## MATERIALS REQUIRED BUT NOT PROVIDED

- Precision pipette (2  $\mu$ L to 200  $\mu$ L) for making and dispensing dilutions
- Test tubes
- Microtitre washer/aspirator
- Distilled or Deionized H<sub>2</sub>O
- Microtitre Plate reader
- Assorted glassware for the preparation of reagents and buffer solutions
- Timer

## ASSAY PROTOCOL

### DILUTION OF SAMPLES

The assay for quantification of IgG1 in samples requires that each test sample be diluted before use. For a single step determination a dilution of 1/50,000 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 1/50,000 dilution of sample, transfer 5  $\mu$ L of sample to 995  $\mu$ L of 1X diluent. This gives you a 1/200 dilution. Next, dilute the 1/200 samples by transferring 4  $\mu$ L, to 996  $\mu$ L of 1X diluent. You now have a 1/50,000 dilution of your sample. Mix thoroughly at each stage.

## PROCEDURE

1. Bring all reagents to room temperature before use.

## 2. Pipette 100 $\mu$ L of

- Standard 0 (0.0 ng/ml) in duplicate
- Standard 1 (6.25 ng/ml) in duplicate
- Standard 2 (12.5 ng/ml) in duplicate
- Standard 3 (25 ng/ml) in duplicate
- Standard 4 (50 ng/ml) in duplicate
- Standard 5 (100 ng/ml) in duplicate
- Standard 6 (200 ng/ml) in duplicate

3. Pipette 100  $\mu$ L of sample (in duplicate) into pre designated wells.

4. Incubate the micro titer plate at room temperature for sixty (60  $\pm$  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  $\mu$ L of appropriately diluted Enzyme-Antibody Conjugate to each well. Incubate at room temperature for twenty (20  $\pm$  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  $\mu$ 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  $\mu$ 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 OF THE FINAL REACTION MIXTURE

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 IgG1 concentration in original samples.

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.

#### **LIMITATION OF THE PROCEDURE**

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

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

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