Version 27-05/24

User's Manual

Mouse Anti-Anthrax PA83 IgG ELISA Kit

REF **DEIASL261**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

This kit is intended to detect anti-Anthrax PA83 IgG antibody in mouse serum or plasma post vaccination.

Principles of Testing

The Mouse anti-PA83 IgG ELISA kit is based on the binding of mouse anti-PA83 IgG in samples to PA83 im mobilized on the microwells, and anti-PA83 IgG antibody is detected by anti-Mouse IgG-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-PA83 IgG present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450 nm is then measured using an ELISA microwell reader. The activity of mouse IgG antibody in samples is calculated relative to anti-Anthrax PA83 calibrators.

Reagents And Materials Provided

1. **PA83 Microwell Strip Plate**: 8-well strips (12). Coated with PA83, and post-coated with stabilizers.
2. **Anti-PA83 Calibrators**: 10 U/ml 20 U/ml 40 U/ml 80 U/ml 160 U/ml, 0.65 m each. Five (5) vials, each containing anti-PA83 IgG levels in arbitrary activity Units; diluted in buffer with protein, detergents and antimicrobial as stabilizers.
3. **Mouse Anti-PA83 Positive Control**: 0.65 ml. Mouse IgG reactive with PA83. Signal > 0.5 net OD.
4. **Wash Solution Concentrate (100×)**: 10 ml. Dilute the entire volume 10 ml + 990 ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temperature until kit is used entirely.
5. **Sample Diluent Concentrate (20×)**: 10 ml. Dilute the entire volume, 10 ml + 190 ml with distilled or deionized water into a clean stock bottle. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.
6. **Anti-Mouse IgG- HRP Conjugate Concentrate (100×)**: 0.15 ml. Peroxidase conjugated anti-Mouse IgG in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10 µl of concentrate to 1ml of Working Sample Diluent is sufficient for 1 8-well strip. Use within the working day and discard. Return 100× to 2-8°C storage.
7. **TMB Substrate**: 12 ml. Chromogenic substrate for HRP containing TMB and peroxide.
8. **Stop Solution**: 12 ml. Dilute sulfuric acid.

Materials Required But Not Supplied

1. Pipettors and pipettes that deliver 100 µl and 1-10 ml. A multi-channel pipettor is recom mended.
2. Disposable glass or plastic 5-15 ml tubes for diluting samples and Anti-Mouse IgG HRP Concentrate.
3. Graduated cylinder to dilute Wash Concentrate; 0.2 to 1 L.
4. Stock bottle to store diluted Wash Solution; 0.2 to 1 L.

5. Distilled or deionized water to dilute reagent concentrates.
6. Microwell plate reader at 450 nm wavelength.

Storage

Store at 2 - 8°C. Do not use the kit beyond the expiration date.

Specimen Collection And Preparation

Culture medium, serum and other biological fluids may be used as samples with proper dilution to avoid solution matrix interference. For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent. If samples will not be assayed immediately, store refrigerated for up to a few weeks, or frozen for longterm storage. Avoid freeze-thaw cycles.

Assay Design

1. Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be < 0.5 OD. This is usually 1/100 or greater dilution for monkey sera with normal levels of IgG and IgM.
2. Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required (See Method A,B). Blank OD should be < 0.3.
3. Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision. Reading values off a Calibrator curve, Method C, has limitations.
4. Run the Mouse anti-PA83 Positive Control. A net OD value greater than 0.5 indicates proper assay performance.
5. Run a range of sample dilutions for expected higher positives that allows calculation of antibody Titer (when specific titer is at least 4-fold higher than non-immune). See Method D.
6. Run samples in duplicate if used for quantitation; non-immunes that are significantly lower than immunes may be run in singlicate. The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

Plate Preparation

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

1. Determine the number of wells for the assay run. Duplicates are recom mended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.
2. Remove the appropriate number of microwell strips from the pouch and return unused strips to the pouch. Reseal the pouch and store refrigerated.
3. Add 200-300 µl Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. **1st Incubation [100 µl - 60 min; 4 washes]**
   - Add 100 µl of calibrators, samples and controls each to predetermined wells.
   - Tap the plate gently to mix reagents and incubate for 60 minutes.
   - Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.
2. **2nd Incubation [100 µl - 30 min; 5 washes]**
   - Add 100ul of diluted Anti-Mouse IgG HRP to each well.
   - Incubate for 30 minutes.
   - Wash wells 5 times as in step 2.
3. **Substrate Incubation [100 µl - 15 min]**
   - Add 100 µl TMB Substrate to each well. The liquid in the wells will begin to turn blue.
   - Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If your microplate reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. **Stop Step [Stop: 100 µl]**
   - Add 100 µl of Stop Solution to each well.
   - Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.
5. **Absorbance Reading**
   - Use any com mercially available microplate reader capable of reading at 450 nm wavelength. Use a program suitable for obtaining OD readings, and data calculations if available.
   - Read absorbance of the entire plate at 450 nm using a single wavelength within 30 minutes after Stop Solution addition. If available, program to subtract OD at 630 nm to normalize well background.

Calculation

Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine Positive Immune and Negative Non-immune, and to Quantitate positive antibody levels.

Method A

Antibody Activity [ELISA Signal & Sample Dilution]

Represent data as net OD units (A450 signal; blank subtracted) ÷ dilution = Total Activity Units.

A Calibrator value in the mid-OD range (e.g., 40 U/ml) can be used to normalize inter-assay values.

Method B.

Positive Index

Experimental sample values may be expressed relative to the values of Control or Non-immune samples, by calculation of a Positive Index. One typical method is as follows:

1. Calculate the net OD mean + 2 SD of the Control/Non-immune samples = Positive Index.
2. Divide each sample net OD by the Positive Index. Values above 1.0 are a measure of Positive Antibody Activity; below 1.0 are Negative for antibody.

A sample value would be Positive if significantly above the value of the pre-immune serum sample or a suitably determined nonimmune panel or pool of samples, tested at the same sample dilution. This calculation quantifies the positive Antibody Activity level.

	Assay Net OD		Calculated Antibody Activity	
Sample	Control	Exptl	Control	Exptl
1	0.243	2.358	0.49	4.79
2	0.351	0.597	0.71	1.21
3	0.286	1.421	0.58	2.89
4	0.357	1.268	0.73	2.58
5	0.512	0.857	1.04	1.74
6	0.342	1.296	0.70	2.63
7	0.298	0.608	0.61	1.24
8	0.285	0.369	0.58	0.75
9	0.157	0.864	0.32	1.76
10	0.187	0.543	0.38	1.10
Mean	0.302			
SD	0.095			
Mean +2 SD	0.492 = Positive Index			

Method C

Use of a Calibrator Curve

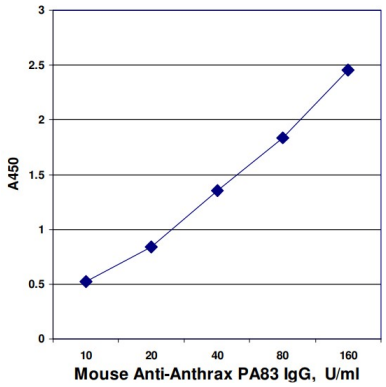
When the dilution curves of samples are parallel to the Calibrator curve, the anti-PA83 activity units may be determined by interpolation from the Calibrator curve. The results may be calculated using any immunoassay software package. If software is not available, anti-PA83 activity concentrations may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the calibrators (y-axis) against the concentration (U/ml) of anti-PA83 (x-axis). Draw the best fit curve through these points to construct the calibrator curve. A point-to-point construction is most common and reliable.
3. The anti-PA83 activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. Samples producing signals higher than the 160 U/ml calibrator should be further diluted and re-assayed.

Typical Results:

Wells	Calibrators		A450 nm
A1,2	Negative Diluent Blank		0.21
B1,2	10 U/m	Calibrator	0.52
C1,2	20 U/m	Calibrator	0.84
D1,2	40 U/m	Calibrator	1.35
E1,2	80 U/m	Calibrator	1.84
F1,2	160 U/m	Calibrator	2.45
G1,2	Sample	1:100	1.45

Sample Result: 46 U/ml x 100 dilution = 4.6 kU/ml



Method D

Titers from Sample Dilution Curves

The titer of antibody activity calculated from a dilution curve of each sample is recommended as the most accurate quantitative method. Best precision can be obtained using the following guidelines:

1. Use an OD value Index in the mid-range of the assay (2.0 - 0.5 OD); this provides the best sensitivity and reproducibility for comparing experimental groups and replicates. An arbitrary 1.0 OD is commonly used.
2. Prepare serial dilutions of each sample to provide a series that will produce signals higher and lower than the selected index. With accurate diluting, duplicates may not be required if at least 4 dilutions are run per sample.
3. A 5-fold dilution scheme is useful to efficiently cover a wide range which produces ODs both above and below 1.0 OD. The dilution scheme can be tightened to 3-fold or 2-fold for more precise comparative data.
4. A Calibrator value in the mid-OD range (e.g., 40 U/ml) can be used to normalize inter-assay values.

Calculations

1. On a log scale of inverse of Sample Dilution as the x-axis, plot the OD values of the two dilutions of each positive sample having ODs above and below the OD value of the Index (arbitrary or selected Calibrator).
2. From a point-to-point line drawn between the two sample ODs, read the dilution value (x-axis) corresponding to the OD of the selected Index = Total IgG Antibody Activity Units

Sensitivity

The PA83 coating level, HRP conjugate concentration and Low NSB Sample Diluent are optimized to differentiate anti-PA83 IgG from background (non-antibody) signal with mouse serum samples diluted 1:100.

Specificity

Recombinant Anthrax PA83 is used to coat the microwells; thus the assay is specific for antibodies directed to PA83. The anti-Mouse IgG HRP conjugate reacts specifically with mouse IgG class antibodies that bind to PA83 on the plate. IgA, IgM and IgE antibody would not be measured above background signals.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains dilute sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Limitations

Quantitation of Antibody in a Sample

The ELISA measures anti-PA83 activity, a combination of antibody concentration and avidity for the PA83 antigens. Antibodies with substantially different total Ig concentrations may display similar antiPA83 activities, due to differences in avidity. The quantitation or activity of the samples is, therefore, appropriately expressed in activity Units (titer), rather than mass units of Ig (e.g., ug/ml).

Calibrator Curve Quantitation

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in PA83 avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 40 U/ml Calibrator, or another Calibrator in the kit (see Calculation).