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User's Manual

Human Dimethyl Phthalate (DMP) ELISA Kit

REF **DEIANJ02**

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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

The ELISA (Enzyme-Linked Immunosorbent Assay) kit is an enzyme-linked immunosorbent assay for the quantitative measurement of samples in serum, plasma, cell culture supernatants and urine.

Principles of Testing

This DMP ELISA is a quantitative competitive immunoassay. The microtiter plate provided is coated with an DMP-specific antibody. Standards or experimental samples are co-incubated in wells along with a DMP-HRP conjugate. DMP in standards or samples competes with DMP-HRP conjugate for binding to the plate bound antibody. Higher levels of DMP from standards or samples leads to decreased DMP-HRP conjugate binding and reduced signal. Captured DMP-HRP is quantitatively detected by incubation with HRP substrates (solutions A and B). Binding of the DMP-HRP is visualized by production of colorimetric reaction products that can be quantitatively measured by absorbance at 450nm.

Reagents And Materials Provided

All reagents must be stored at 2-8° C. Refer to the expiration date on the label.

1. Microplate, 96 strip wells
2. Standard A 0ng/ml, 1 mL
3. Standard B 50ng/ml, 1mL
4. Standard C 100ng/ml, 1 mL
5. Standard D 250ng/ml, 1 mL
6. Standard E 500ng/ml, 1mL
7. Standard F 1000ng/ml, 1mL
8. Substrate A, 1 vial, 6.0mL
9. Substrate B, 1 vial, 6.0 mL
10. Stop Solution, 1 vial, 6.0 mL
11. Enzyme Solution, 1 vial, 6.0mL
12. Balance Solution, 1 vial, 1 mL
13. Wash Solution (25×), 1 vial, 50 mL

Storage

Store all reagents at 2-8°C

Specimen Collection And Preparation

GENERAL CONSIDERATIONS

1. Samples should be handled following standard practices to minimize degradation or denaturation. Avoid multiple freeze-thaw cycles or high temperatures. For long term storage, maintain samples at temperatures that minimize degradation or denaturation.
2. Without prior knowledge of the analyte concentration, determining the amount of sample required for robust detection is difficult. Therefore, we recommend that enough sample be collected to allow for multiple dilutions to be assayed.
3. Experimental variation between wells is to be expected. We recommend assays be performed using at least two wells for every sample or standard. Readings of duplicate wells should be averaged.
4. Two blank wells containing sample buffer should be included to determine background.

Reagent Preparation

1. Bring all kit components and samples to room temperature before use
2. Bring microtiter plate to room temperature before opening. Remove the desired number of well strips and immediately reseal and store at 2-8°C.
3. Dispense 10 µL of BALANCE SOLUTION into 100 µL experimental samples. **NOTE: This step is required only when the sample is cell culture supernatant, body fluid or tissue homogenate.**
4. Dilute 40 mL of WASH SOLUTION concentrate (25×) with 960 mL of deionized or distilled water. If crystals have formed in the concentrate warm to room temperature and mix to dissolve.

Assay Procedure

1. Add 100 µL of SAMPLE or STANDARD to the appropriate number of wells in the supplied microtiter plate. Note that wells have been pre-blocked and no additional blocking steps are required. Add 100µL of PBS (pH7.0-7.2) or water to the blank well.
2. Add 50 µL of Enzyme Solution to each well (but NoT blank well) in the supplied Plate and mix well.
3. Cover and incubate 1 hour at 37°C in a humid chamber.
4. Wash each well 5 times with 300-400 µL 1× WASH SOLUTION per well. After the last wash invert the plate and blot dry by tapping on absorbent paper: **Note: Hold the sides of the plate frame firmly when washing to assure that all strips remain securely in the frame. Complete removal of the liquid at each step is essential for good performance.**
5. Add 50 µL SUBSTRATE A to each well followed by addition of 50 µL SUBSTRATE B. Cover and incubate 10-15 minutes at room temperature. SUBSTRATE is light sensitive. Keep out of direct sunlight or cover with foil.
6. Add 50 µL of STOP SOLUTION to each well and mix well.
7. Immediately read the optical density (O.D.) at 450 nm.
8. Subtract the mean blank value from each SAMPLE or STANDARD value and calculate the mean for duplicate (or greater) wells.
9. Construct the standard curve using graph paper or statistical software.

Note:

1. When not in use, kit components should be refrigerated. All reagents should be warmed to room temperature before use.

2. Samples should be collected in pyrogen/endotoxin-free tubes.
3. When possible, avoid use of hemolyzed or lipemic sera. Centrifuge or filter samples if particulate matter is present.
4. It is recommended that all standards, controls, and samples be run at least in duplicate.
5. To ensure equal incubation times maintain a consistent order of addition from well-to-well when pipetting reagents.
6. Cover or cap all reagents when not in use.
7. Do not mix or interchange different reagent lots from various kit lots.
8. Do not use reagents after the kit expiration date.
9. Determine absorbance within 2 hours of assay completion.
10. The provided controls should be run with every assay.
11. Substrate B is light sensitive. Avoid prolonged exposure to light. Substrate B will discolor metals so contact should be avoided.
12. Incomplete washing will adversely affect the test outcome. All washing must be performed with the provided Wash Solution.
13. Washing can be performed using a squirt bottle and filling all wells to the top.
14. Do not mix reagents from different lots. It is recommended that assays be performed at least in duplicate. Standards and samples must be assayed at the same time.

Calculation

Calculation of competitive ELISA results

After obtaining raw data from the ELISA reader, the ELISA results are ready for statistical analysis. We suggest using an ELISA data analysis software for the analysis, such as CurveExpert 1.4 or GraphPad Prism. Microsoft Excel can also be a useful tool to analyze the data. Below is an example we use CurveExpert 1.4 to process the raw data.

1. Enter ELISA Data Into Software

Enter the standard concentration in the x-axis column and the corresponding OD values in the y-axis column.

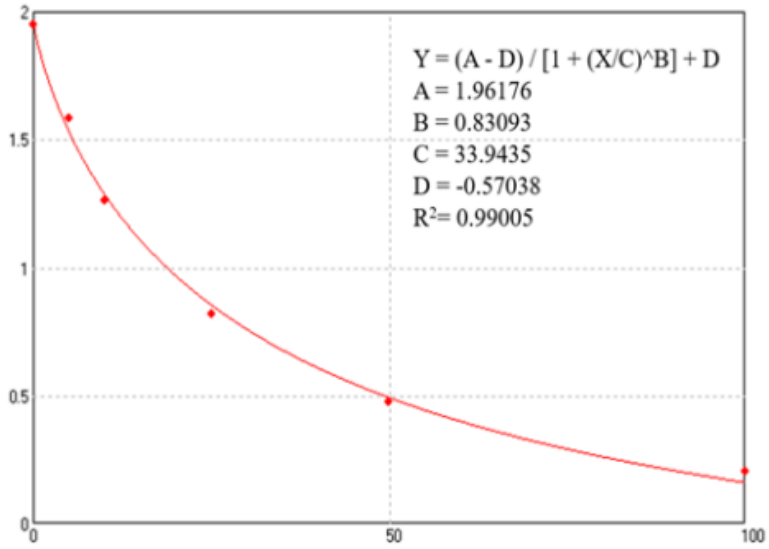
2. Select The Best Fitting Curve

Our lab and most companies generally recommend using a 4-parameter algorithm for the best standard curve fit. Users are welcome to try other models for calculation. The ideal curve to be picked should rise smoothly and closely resemble a straight line and the equation should be with the higher R value.

3. Calculate Target Protein Concentration

The calculation can be performed in the software. If the samples were diluted before the ELISA, make sure to multiply the computed sample concentrations by the sample dilution factor.

Typical Standard Curve



Sensitivity

The sensitivity of this assay should be approximately 1.0ng/ml.

Specificity

This assay has high sensitivity and excellent specificity for the detection of DMP. No significant cross-reactivity or interference between DMP and any homologous proteins assayed has been observed. Species cross-reactivity has not been specifically determined.

Precautions

1. This kit contains 3,3',5,5'-Tetramethylbenzidine (TMB) in Substrate B. TMB, present at levels greater than or equal to 0.1% is NOT identified as a carcinogen or potential carcinogen by OSHA. TMB may cause irritation to skin and eyes. Please wear appropriate personal protective equipment, including gloves, safety glasses, and lab coats when handling.
2. The Stop Solution provided in the kit is an acidic solution. Please wear appropriate personal protective equipment (gloves, safety glasses, lab coat) when handling this and all kit components.
3. All blood components and biological materials should be handled as potentially hazardous. Follow universal precautions as established by the Centers for Disease Control and Prevention and by the Occupational Safety and Health Administration when handling and disposing of infectious agents.
4. All waste must be disposed of in accordance with all applicable local, state, and federal regulations.