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User's Manual

Mouse Adenovirus K87 ELISA Kit

REF **DEIASL109**

Σ **96T**

RUO

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The kit is intended for the detection of antibodies to Murine Adenovirus K87 (MAD-2) in Mouse Sera by ELISA.

Principles of Testing

The micro test wells are coated in alternating columns with Murine Adenovirus K87 (MAD-2) Antigen and Control-Antigen. During the first incubation with the diluted specimen, any antibodies that are reactive with the MAD-2 Antigen or Control-Antigen will bind to the coated wells. After washing to remove the rest of the sample, the Enzyme Conjugate is added. If antibodies have been bound to the wells, the Enzyme Conjugate will then bind to these antibodies. After another series of washes, a chromogen is added. If the Enzyme Conjugate is present, the peroxidase will catalyze a reaction that consumes the peroxide and turns the chromogen from clear to blue. Addition of the Stop Solution ends the reaction and turns the blue color to a bright yellow color. The reaction is then read with an ELISA reader and an Index Value is calculated from the Differential OD (Antigen well OD minus Control-Antigen well OD) of the specimen.

Reagents And Materials Provided

1. Test Strips: Two 96-breakaway well Microplates in holders coated in alternating columns with MAD-2 Antigen and Control-Antigen. The Antigen wells are ringed in black and the Control-Antigen wells are ringed in red.
2. Enzyme Conjugate: One 11ml bottle of anti-mouse IgG conjugated to peroxidase
3. Reactive Control Serum: One 1ml vial of diluted reactive serum
4. Negative Control Serum: One 1ml vial of diluted negative serum
5. Calibrator: One 100µl vial of reactive serum
6. Chromogen: One 11ml bottle of TMB
7. Wash Buffer: Three 25ml bottles of concentrated (20X) buffer with surfactant
8. Dilution Buffer: Two 30ml bottles of buffered protein solution
9. Stop Solution: One 11ml bottle of 1M Phosphoric Acid

Materials Required But Not Supplied

1. Pipettes capable of delivering 5µl, 50µl, and 250µl
2. Squeeze bottle for washing strips or automated plate washer (see procedural notes)
3. Distilled or reagent grade water and graduated cylinder
4. Tubes for sample and calibrator dilution
5. Absorbent paper
6. A dual wavelength (bichromatic) ELISA plate reader with a 450nm and a 620 to 650nm filter. If a bichromatic reader is not available, a single wavelength ELISA reader with a 450nm filter can be used.

Storage

Store the reagents, strips and bottled components between 2 - 8°C.

The diluted wash buffer may be stored at room temperature for up to 180 days, but do not use if it becomes cloudy. Do not add fresh buffer to old buffer.

Specimen Collection And Preparation

This test utilizes the specimen's serum: coagulate the blood and remove the serum. The use of "bloody" sera is contra-indicated. Serum samples should be refrigerated as soon as possible after collection and tested within 48 hours. If the specimen is not to be tested within 48 hours after collection, the serum sample should be frozen at 0°C or lower.

Avoid repeated freezing and thawing of samples.

Vortex (mix well) samples, controls, and calibrator before using.

Do not use pooled specimens as this will adversely affect the performance of the assay.

Test samples and the calibrator are diluted 1:51 in the dilution buffer (5µl of sera + 250µl of dilution buffer)

The Negative and Reactive Controls are ready to use; do not dilute.

All Reagents must be at room temperature before beginning the assay.

Reagent Preparation

1. Bring one bottle of the 20x Wash Solution to 500ml with distilled water. MIX WELL
2. Controls are ready to use, do not dilute.
3. Set up calibrator and specimen dilution tubes according to the plate map.
4. Prepare 1:51 dilution of the calibrator and specimens as follows:

Add 250µl of Dilution Buffer to all dilution tubes.

Add 5µl of each specimen or calibrator to appropriate tube. MIX WELL

Assay Procedure

1. Prepare the plate map. The columns alternate between black-ringed Antigen wells and red-ringed Control-Antigen wells. Each Control, Calibrator, and specimen requires two wells: an Antigen coated well and a Control-Antigen coated well.

Well Location	Identification
A1 & A2	Reactive Control
B1 & B2	Negative Control
C1 & C2	Calibrator
D1 & D2	Specimen #1
E1 & E2	Specimen #2
F1 & F2	Etc.

2. Break-off the required number of Antigen and Control-Antigen wells and place into the plate holder. (Unused wells must be kept sealed in a dry environment).
3. Add 50µl of the pos and neg controls to the appropriate wells.
4. Transfer 50µl of each specimen and calibrator dilution tube to the appropriate wells.
5. Incubate at room temperature for 30 MINUTES.
6. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
7. Repeat the above step four more times for a total of 5 WASHES. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.
8. Add 50µl (1 drop) of conjugate to each well. Incubate at room temperature for 30 MINUTES.
9. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
10. Repeat the above step four more times for a total of 5 WASHES. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.
11. Add 50µl (1 drop) of CHROMOGEN to each well. Incubate at room temperature for 10 MINUTES. DO NOT DUMP AFTER THIS INCUBATION PERIOD.
12. Add 50µl (1 drop) of STOP SOLUTION to each well. Brace the plate with one hand and gently tap along the opposite side of the plate with the other to evenly distribute the Stop Solution in each well.
13. Read the plate at bichromatic 450/620-650nm wavelengths (or a single wavelength of 450nm) within 30 minutes of adding the Stop Solution.

Quality Control

The use of controls allows validation of the test. The results should not be used if a control, or the calibrator, is out of range. A run is valid if all three of the following conditions are met:

Negative Control - Index Value of less than 0.60

Reactive Control - Index Value between 1.50 and 6.50

Calibrator - The Differential OD must be greater than 0.00

Interpretation Of Results

Each Control, Calibrator, and Specimen has an OD result from the Antigen Well and the Control-Antigen Well. The OD of the Control-AntigenWell is subtracted from the OD of the Antigen Well to yield the Differential OD.

For Example:

	Antigen Well OD	Control-Antigen Well OD	Differential OD
Negative Control:	0.12	0.04	0.08
Reactive Control:	1.82	0.03	1.79
Calibrator:	0.38	0.10	0.28
Specimen 1:	1.10	0.19	0.91
Specimen 2:	0.25	0.02	0.23

The Index Value of the controls and specimens is obtained by dividing the Differential OD of the specimen or control by the Differential OD of the calibrator.

Calculation of the Example:

Differential OD of the Calibrator	0.28		
Index Value of Negative Control	0.08/0.28	0.29 Index	Valid
Index Value of Reactive Control	1.79/0.28	6.39 Index	Valid
Index Value of Specimen 1	0.91/0.28	3.25 Index	Positive
Index Value of Specimen 2	0.23/0.28	0.82 Index	Negative

An Index Value of 1.0 or greater is considered Positive.

An Index Value of less than 1.0 is considered Negative.

Negative Index Values are considered to be 0.00.

Evaluation

The normal value is Negative. Studies have shown that antibodies may take up to 21 days to appear after exposure; therefore, Negative specimen results should be reviewed in relation to a possible exposure date. All Positive specimen results should be confirmed by an alternate method.

Precautions

1. The kit Calibrator and Controls must be included on each plate tested.
2. Allow all reagents and samples to come to room temperature before testing. It is normal for the concentrated wash buffer to crystallize when cold. The crystals will re-dissolve once the solution returns to room temperature.
3. Do not use reagents beyond the expiration date printed on the label.
4. The dropper tips are removable from the reagent bottles to allow pipetting of reagents.
5. Do not inter-mix conjugates, calibrators, controls, or coated plates between different kits or different lot numbers of the same kit: these components are balanced to work together as a unit. The wash buffer, substrate, stop reagent, and dilution buffer are universal reagents and can be inter-changed between all Creative Diagnostics ELISA kits.
6. There are several types of automated plate washers available. If using an automated plate washer, you will need to validate the performance of your particular washer on the Creative Diagnostics assays. This can be done as simply as performing a side-by-side comparison of results achieved by manual washing versus automated washing. At the conclusion of each wash cycle, invert the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.
7. This product is warranted to perform as described in the labeling provided that: the product is stored and used as directed; used before the expiration dating; and adequate quality control is performed. No other warranty is implied, nor are we liable for any consequential damages arising out of the aforesaid warranty.