Lymphocytic Choriomeningitis Virus (Mouse Sera)

Catalog # SMART-M15

For the detection of antibodies to Lymphocytic Choriomeningitis Virus (LCM) in Mouse Sera by ELISA

For Use On Research Animals Only

ASSAY PRINCIPLE

The micro test wells are coated in alternating columns with Lymphocytic Choriomeningitis Virus (LCM) Antigen and Control-Antigen. During the first incubation with the diluted specimen, any antibodies that are reactive with the LCM 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 PROVIDED

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

REAGENT STORAGE

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

SERUM COLLECTION AND HANDLING

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.

MATERIALS REQUIRED BUT NOT PROVIDED

Pipettes capable of delivering 5µl, 50µl, and 250µl
Squeeze bottle for washing strips or automated plate washer (see procedural notes)
Distilled or reagent grade water and graduated cylinder
Tubes for sample and calibrator dilution
Absorbent paper
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.

PROCEDURE

See attached procedure guide. All procedures and reagents are at room temperature (15 – 25° C).

READING RESULTS

ELISA Reader: Zero reader on air. Set for bichromatic readings at 450/620-650nm or for a single wavelength of 450nm
Positive – Index Value of 1.0 or greater
Negative – Index Value of less than 1.0

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-Antigen Well 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**

If using the SMART-Calc Excel Program, just enter the OD value from the reader into the appropriate well on the Excel grid and SMART-Calc will perform all of the calculations automatically.

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

EXPECTED VALUES

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.

TROUBLESHOOTING

Negative Control has an Index greater than 0.59

Suggestion: wash more vigorously. Remove excessive liquid from the wells by slapping plate, well side down, against an absorbent towel. Do not allow test wells to dry out.

Reactive Control has an Index Value of less than 1.50 or more than 6.50

Suggestion: check the three "T's": Time, Temperature, and Technique. Time: insure that the timing on the incubation stages is adhered to. Temperature: temperatures above 25^{o}C may adversely affect the assay; Technique: check all pipettes to insure that they are properly delivering the correct volume to produce a 1:51 dilution of the calibrator and specimens. Also check your wash procedure to insure vigorous washing and removal of excess wash buffer.

PROCEDURAL NOTES

The kit Calibrator and Controls must be included on each plate tested.

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.

Do not use reagents beyond the expiration date printed on the label.

The dropper tips are removable from the reagent bottles to allow pipetting of reagents.

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 SMART ELISA kits.

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 SMART 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.

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.

Biotech Trading Partners, Inc

1042B N El Camino Real
Suite 341
Encinitas, CA 92024
Phone: 1-760-578-6176
Fax: 1-267-295-8218
Email: info@biotechtradingpartners.com
Web Site: www.biotechtradingpartners.com



A graduate of the SMART program

EIA PROCEDURE GUIDE

Lymphocytic Choriomeningitis Virus (Mouse Sera), Catalog #SMART-M15

INITIAL SETUP

1. Bring one bottle of the 20x Wash Solution to 500ml with distilled water. **MIX WELL**
2. 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.

3. Controls are ready to use, do not dilute
4. Set up calibrator and specimen dilution tubes according to the plate map.
5. 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**

SERUM INCUBATION STAGE

6. 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)
7. Add 50µl of the pos and neg controls to the appropriate wells.
8. Transfer 50µl of each specimen and calibrator dilution tube to the appropriate wells.
9. Incubate at room temperature for **30 MINUTES.**

WASH STAGE

10. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
11. Repeat the above step four more times for a total of **5 WASHES.**
12. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.

CONJUGATE INCUBATION STAGE

13. Add 50µl (1 drop) of conjugate to each well.
14. Incubate at room temperature for **30 MINUTES.**

WASH STAGE

15. After 30 minutes, dump the tray. Refill each well to the top with wash buffer and dump.
16. Repeat the above step four more times for a total of **5 WASHES.**
17. After the last wash, dump the tray and slap the wells hard against a paper towel 3-4 times to remove excess buffer.

SUBSTRATE STAGE

18. Add 50µl (1 drop) of CHROMOGEN to each well.
19. Incubate at room temperature for **10 MINUTES.**
20. **DO NOT DUMP AFTER THIS INCUBATION PERIOD.**

STOP STAGE

21. Add 50µl (1 drop) of STOP SOLUTION to each well.
22. 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.
23. Read the plate at bichromatic 450/620-650nm wavelengths (or a single wavelength of 450nm) within 30 minutes of adding the Stop Solution.

SMART EIA PLATE MAP

ASSAY NAME: _____ **DATE:**_____

LOT #: _____ **PLATE:** _____

	Antigen 1	Control 2	Antigen 3	Control 4	Antigen 5	Control 6	Antigen 7	Control 8	Antigen 9	Control 10	Antigen 11	Control 12
A	REACTIVE CONTROL		SPECIMEN #6		SPECIMEN #14		SPECIMEN #22		SPECIMEN #30		SPECIMEN #38	
B	NEGATIVE CONTROL		SPECIMEN #7		SPECIMEN #15		SPECIMEN #23		SPECIMEN #31		SPECIMEN #39	
C	CALIBRATOR		SPECIMEN #8		SPECIMEN #16		SPECIMEN #24		SPECIMEN #32		SPECIMEN #40	
D	SPECIMEN #1		SPECIMEN #9		SPECIMEN #17		SPECIMEN #25		SPECIMEN #33		SPECIMEN #41	
E	SPECIMEN #2		SPECIMEN #10		SPECIMEN #18		SPECIMEN #26		SPECIMEN #34		SPECIMEN #42	
F	SPECIMEN #3		SPECIMEN #11		SPECIMEN #19		SPECIMEN #27		SPECIMEN #35		SPECIMEN #43	
G	SPECIMEN #4		SPECIMEN #12		SPECIMEN #20		SPECIMEN #28		SPECIMEN #36		SPECIMEN #44	
H	SPECIMEN #5		SPECIMEN #13		SPECIMEN #21		SPECIMEN #29		SPECIMEN #37		SPECIMEN #45	

Multi-Species EIA Serology Assays

BTP-96200	QnE Hyaluronic Acid (HA) Quantitative ELISA assay

Mouse ELISA Serology Assays

SMART-M10	MVM, Minute Virus of Mice
SMART-M11	Sendai Virus
SMART-M12	PVM, Pneumonia Virus of Mice
SMART-M13	REO-3, REO Virus Type 3
SMART-M14	TMEV (GDVII), Theiler's Murine Encephalomyelitis Virus
SMART-M15	LCM, Lymphocytic Choriomeningitis Virus
SMART-M16	Ectromelia Virus
SMART-M17	MHV, Mouse Hepatitis Virus
SMART-M18	Polyoma Virus
SMART-M19	EDIM, Epizootic Diarrhea of Infant Mice, (Mouse Rotavirus)
SMART-M23	K, Mouse Pneumonitis Virus
SMART-M24	MCMV, Mouse Cytomegalovirus
SMART-M25	MAD 1, Murine Adenovirus FL
SMART-M26	MAD 2, Murine Adenovirus K87
SMART-M27	MPUL, Mycoplasma pulmonis
SMART-M28	MPV (*r*VP2), Mouse Parvovirus
SMART-M30	CARB, Cilia-Associated Respiratory Bacillus
SMART-M31	ECUN, *Encephalitozoon cuniculi*
SMART-M32	CPIL, Tyzzer's Disease (*Clostridium piliforme*)
SMART-M33	Hantaan Virus
SMART-M35	Murine Norovirus

Rat ELISA Serology Assays

SMART-R11	Sendai Virus
SMART-R12	PVM, Pneumonia Virus of Mice
SMART-R13	REO-3, REO Virus Type 3
SMART-R14	TMEV (GDVII), Theiler's Murine Encephalomyelitis Virus
SMART-R15	LCM, Lymphocytic Choriomeningitis Virus
SMART-R20	SDAV/RCV, Rat Coronavirus/Sialodacroadenitis Virus
SMART-R21	KRV, Kilham Rat Virus
SMART-R22	H1, Toolan's Virus
SMART-R25	MAD-1,2, Murine Adenovirus FL/K87
SMART-R27	MPUL, Mycoplasma pulmonis
SMART-R29	RPV (*r*VP2) Rat Parvovirus
SMART-R30	CARB, Cilia-Associated Respiratory Bacillus
SMART-R31	ECUN, *Encephalitozoon cuniculi*
SMART-R32	CPIL, Tyzzer's Disease (*Clostridium piliforme*)
SMART-R33	Hantaan Virus

Non-Human Primate ELISA Serology Assays

4810	Herpes B-Virus
4811	Simian Immunodeficiency Virus (SIV)
4812	Simian Retrovirus (SRV)
4813	Simian T-Lymphotropic Virus (STLV)
4814	Simian Measles Virus

Multiple Analyte SPOT Serology Assays

MOUSE: SMART-SPT9M
Simultaneous & discrete detection of antibodies to EDIM; Mouse Hepatitis Virus; Mycoplasma pulmonis; Mouse Parvovirus, Minute Virus of Mouse; Pneumonia Virus of Mice; REO-3; Sendai, and TMEV (GD7) in mouse serum

MOUSE: SMART-SPT6EM
Simultaneous & discrete detection of antibodies to Clostridium piliforme; Ectromelia Virus; LCM; Hantaan Virus; Mouse Adenovirus Fl/K87; Mouse Cytomegalovirus in mouse serum

RAT: SMART-SPT8R
Simultaneous & discrete detection of antibodies to KRV; Mycoplasma pulmonis; Pneumonia Virus of Mice; SDAV/RCV; REO-3; Rat Parvovirus; Sendai, and TMEV (GD7) in rat serum.

NON-HUMAN PRIMATE SIMIAN-SPT5S
Simultaneous & discrete detection of antibodies to Herpes B-Virus; SIV; STLV; SRV; and Measles in non-human primate serum.