Measles ELISA

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Up-dated by: Date:

Aim

The detection of antibodies (Ab) against Measles virus in the serum of Resus monkeys by a sandwich Enzyme Linked ImmunoSorbent Assay. We received the original protocol from Berna Biotech AG.

Materials

- ELISA plates (NUNC Maxisorp)
- Coating antigen : Measles Virus EIA Bulk, Edmonsten (ATCC VR-24; cat# 7190MB). Use at 1.2 µg/ml in Coating Buffer.
- Coating Buffer : 0.05M Carbonate Buffer pH 9.4 (e.g. 8 ml 0.2M Na_2CO_3 (21.2 g/l) + 17 ml 0.2M $NaHCO_3$ (16.8 g/l) + 75 ml MQ)
- Blocking Buffer : PBS + 10% w/v milkpowder
- Wash Buffer : PBS + 0.05% v/v Tween-20
- Dilution Buffer : PBS pH 7.4 + 0.05% v/v Tween-20
- Positive control serum
- Negative control serum M094
- Conjugate: Goat α Monkey IgG coupled to HRP
- Substrate: ready-to-use TMB plus (KEM EN TEC; cat# 4390A). Bring to RT before use, and protect from light.
- Stopping Solution: 0.2M H_2SO_4 (e.g. 20 ml H_2SO_4 95-97% + 980 ml MQ)
- Plate washer (See protocol ***2.6*** *Plate Washer*)
- Plate reader (See protocol ***2.12*** *Plate Reader Manual*)

Method

1. Coat ELISA plates with 100 µl/well Measles antigen diluted in Coating Buffer.
2. Cover the plate with a seal (or lid) and incubate O/N at 4 ºC.
3. Remove coating from plates by inverting the plates with a vigorous wrist action.
4. Block with 300 µl/well of Blocking Buffer.
5. Cover the plate with a seal and incubate 1 hour at 37 ºC.
6. Wash with plate washer (program 9).
7. Prepare samples and controls: The first dilution of samples and negative control is 1:100 (e.g. 4 µl in 400 µl Dilution Buffer), the positive control starts at 1:400 (e.g. 5 µl in 2 ml Dilution Buffer).
8. Add 100 µl of Dilution Buffer to all wells not filled with the first dilution of the samples and controls.
9. Add 100 µl of each pre diluted sample or control to the first wells of a column and make a serial dilution over 8 wells. The positive control is serial diluted 2-fold over 8 wells, using 100 µl. The samples are serial diluted 3-fold over 4 wells, and the negative control over 3 wells, using 50 µl. The last well of the negative control

is used as blanc (See below for a design of plate format). Final volume in the wells is 100 µl.

10. Cover the plate with a seal and incubate 1 hour at 37 ºC.
11. Wash with plate washer (program 9).
12. Add 100 µl/well conjugate in Dilution Buffer.
13. Cover the plate with a seal and incubate 1 hour at 37 ºC.
14. Wash with plate washer (program 9).
15. Add 100 µl of ready-to-use TMB.
16. Incubate 15 minutes at RT in the dark.
17. Stop the reaction by addition of 50 µl/well of Stopping Solution.
18. Read OD at 450 nm on a Microplate Reader. (Before stopping the reaction the OD can be read at 655 nm.)

Schematic:

Action	Materials	Concentration	Dilute in	Incubate
Coating	Measles Antigen	1.2 µg/ml	Coating buffer	O/N 4 ºC
Blocking	milkpowder	0.1 g/ml (10%)	PBS	1 hr RT
Samples	Positive control	1st dilution 1:400	Dilution Buffer	1 hr 37 ºC
	Negative control	1st dilution 1:100	Dilution Buffer	
	(Pre-) immune sera	1st dilution 1:100	Dilution Buffer	
Conjugate	Go anti Monkey IgG-HRP	1:3000	Dilution buffer	1 hr 37 ºC
Substrate	ready-to-use TMB plus			15 min RT
Stopping Solution	95-97% H_2SO_4	0.02M	MQ	

Possible plate format

	1	2	3	4	5	6	7	8	9	10	11	12
A	Sample 01 1:100	Sample 02 1:100	Sample 03 1:100	Sample 04 1:100	Sample 05 1:100	Duplicates of columns 1-5					Positive control 1:400	
B	1:300	1:300	1:300	1:300	1:300						1:800	
C	1:900	1:900	1:900	1:900	1:900						1:1600	
D	1:2700	1:2700	1:2700	1:2700	1:2700						1:3200	
E	Sample 06 1:100	Sample 07 1:100	Sample 08 1:100	Sample 09 1:100	Negative control 1:100	Duplicates of columns 1-5					1:6400	
F	1:300	1:300	1:300	1:300	1:300						1:12800	
G	1:900	1:900	1:900	1:900	1:900						1:25600	
H	1:2700	1:2700	1:2700	1:2700	blanc					bl	1:51200	