



Reagents and supplies for Zika IgM ELISA

Coating buffer:

Carbonate/bicarbonate buffer pH 9.6
1.59g Na₂CO₃ + 2.93g NaHCO₃ diluted in 1L water.

Wash buffer:

Phosphate buffered saline (PBS), 0.05% Tween 20, pH 7.2.
PBS is available in powdered form from multiple commercial sources.

Blocking buffer: PBS/5% milk/ 0.5% Tween 20

Coating antibody:

Goat anti-human IgM
Kirkegaard and Perry Laboratories cat# 01-10-03

Positive control:

6B6C-1 huIgM chimera (CDC) or Zika IgM-positive serum.

Negative control:

Known virus-negative serum

Viral antigen:

Sucrose-acetone extracted suckling mouse brain viral antigens, gamma-irradiated tissue culture antigen, non-infectious, previously titrated, or COS-1 antigen (non-infectious) (CDC)

Normal antigen:

Sucrose-acetone extracted suckling mouse brain antigen, tissue culture antigen, or normal COS-1 antigen (CDC)

Detecting antibody conjugate:

Horseradish peroxidase conjugated monoclonal antibody 6B6C-1 (commercially available from Hennessy Research and InBios. Note: the Hennessy detecting antibody conjugate cannot be used with COS-1 antigens)*.

Substrate:

3,3',5,5' tetramethylbenzidine base (Enhanced K-Blue TMB substrate), Neogen Corp.
Cat# 308175

Stop solution:

TMB Stop Solution KPL Cat# 50-85-05 (75 µl/well) or 1 N H₂SO₄
(50 µl/well)

Plates:

Immulon II HB flat-bottomed 96 well plates
Dynatech Technologies cat# 3455

Microplate washer

Microplate reader with 450 nm filter

Refrigerator

Incubator (37C)
Single and multi-channel pipettors
Reagent reservoirs
Ziploc bags, paper towels

*For updated information regarding conjugates, please refer to LRN guidance.