FLAVIVIRUS SEROLOGICAL PANEL 1-3 PROTOCOL

Catalog # 600-002080, 600-002081

Use

For the detection of antibodies (IgG) to Chikungunya (CHIK), Dengue (DEN), Equine Encephalitis (EEV), St. Louis Encephalitis (ST.LEV), Japanese Encephalitis (JEV), West Nile (WNV), Yellow Fever (YFV) and Zika Virus in human or mouse serum and plasma samples.

Reagents Supplied

Flavivirus Serological Panel 1-3 Beads - Part # 100-001470 (pre-mixed, ready to use)
1 vial containing **1.2 mL** of multiplexed magnetic beads for CHIK-E, DENV1-VLP, DENV2-NS1, DENV3-NS1, DENV4-VLP, EEV, SLEV-NS1, WNV-ENV, WNV-NS1, JEV-ENV, YFV-NS1, YFV-ENV, ZIKA-NS1 and ZIKA- VLP antigens in a proprietary buffered solution with 0.05% ProClin 300.
Or

Flavivirus Simplex Beads- Part (see attached)
1 vial containing **75 µL** of magnetic beads for one of the following CHIK-E, DENV1-VLP, DENV2-NS1, DENV3-NS1, DENV4-VLP, EEV, SLEV-NS1, WNV-ENV, WNV-NS1, JEV-ENV, YFV-NS1, YFV-ENV, ZIKA-NS1 and ZIKA- VLP antigens in a proprietary buffered solution with 0.05% ProClin 300.

Serological Panel IgG Detection(20X) - Part # 200-001440
1 vial containing **0.25 mL** of a 20X stock of anti-IgG-PE diluted in PBS with 0.02% Tween 20 and 0.05% ProClin 300.

Flavivirus Serological Panel 1-3 Standard - Part # 300-001452
2 vials containing a lyophilized mix of IgG positive serum* with 0.05% ProClin 300.

Flavivirus Serological Panel 1-3 Control 1 - Part # 400-001454-1
2 vials containing mid- levels of lyophilized IgG positive serum* with 0.05% ProClin 300.

Flavivirus Serological Panel 1-3 Control 2 - Part # 400-001454-2
2 vials containing high levels of lyophilized IgG positive serum* with 0.05% ProClin 300.

Serological Panel Sample/Standard Diluent - Part # 520-001094
1 bottle containing 40 mL of PBS with 0.05% ProClin 300.

10X Assay Buffer - Part # 530-001031
1 bottle containing 25 mL of 10X PBS, 0.5% Tween 20 and 0.05% ProClin 300.

Serological Panel Bead Diluent – Part # 530-001032 (for dilution of simplex beads)
1 vial containing 1.2mL of a proprietary buffered solution with 0.05% ProClin 300.

Bead Mixing Vial (for use with simplex beads)

Clear Bottom Black Assay Plate

Plate Cover with Foil Sealer

Required Equipment and Materials Not Supplied

- 0.5 mL to 5 mL polypropylene tubes
- Plate washer with magnetic bead separator or manual magnetic bead separator
- Vortex
- Variable volume pipettes and tips (10 µL to 1000 µL)
- Graduated cylinder(s) (50 mL to 1L)
- 1 L bottle
- milliQ H_20 (18.2 MΩ·cm) or equivalent
- Plate shaker (Fisher part #4625Q) or equivalent.
- Luminex®100/200, MAGPIX®, xMAP INTELLIFLEX®, FLEXMAP 3D®, or Bio-Rad® Bio-Plex® system

Storage and Stability

Store kit at 2-8°C. Do not use beyond the expiration date.

Protocol

Note: Protocol is designed for assaying one (1) 96-well plate.

Equipment and Materials Preparation

1. Set up equipment and materials listed above to be accessible throughout the following protocol.
2. **Luminex Settings**
   Note: Refer to the instrument's manual.
   a. Adjust the probe height on the Luminex appropriately for the plate being used.

      (1) See lot-specific certificate of analysis for standard concentrations

	Bead Regions						
Analyte	**CHIK-E**	**DENV1-VLP**	**DENV2-NS1**	**DENV3-NS1**	**DENV4-VLP**	**EEV**	**JEV**
Bead Region	**15**	**25**	**27**	**30**	**36**	**62**	**52**
Analyte	**St. LEV-NS1**	**WNV-ENV**	**WNV-NS1**	**YFV-ENV**	**YFV-NS1**	**ZIKAVLP**	**ZIKA-NS1**
Bead Region	**47**	**48**	**19**	**66**	**22**	**45**	**20**

      (2) Collection parameters
         (a) Sample Volume: 60 µl
         (b) Minimum events: 50 per region
         (c) Flow rate: 60 µL/Min (fast)
         (d) Doublet Discriminator gates: **Use software settings for running MagPlex beads.**
         (e) Collect Median RFU (MFI), low or standard PMT setting is recommended

B. Reagent Preparation

1. Reconstitute Lyophilized Reagents
   a. Reconstitute the following reagents according to the table below.

Reagent	Reconstitute In:	Volume
Standard	milliQ H_20	150 µL
Control 1	milliQ H_20	100 µL
Control 2	milliQ H_20	100 µL

   b. Vortex at a medium setting.
   c. Allow to sit for a minimum of 10 minutes (not to exceed 1 hour).
2. Assay Buffer
   a. Bring the 10X Assay Buffer to room temperature.
   b. Mix by inversion to bring all salts into solution.
   c. Dilute 1-part 10X Assay Buffer with 9 parts of dH_20 (1X Assay Buffer).
   d. Label with preparation date and store at 2-8°C for up to 1 month.
   e. Aliquot approximately 5mL of 1X Assay Buffer for Detection dilution (Step E5)
3. Bead Preparation
   a. Pre-mixed beads are supplied as ready to use.
   b. For individual 20X beads, vortex at medium bead for 10-20 seconds. Mix bead by pipetting up and down and add 60 µL of 20X bead stock to the mixing vial provided and bring final volume to 1.2mL in Bead Diluent (see table below for diluent volume). Mix well.

Plex Size	Bead Diluent µL	Plex Size	Bead Diluent µL	Plex Size	Bead Diluent µL	Plex Size	Bead Diluent µL
1	1140	**6**	840	**11**	540	**16**	240
2	1080	**7**	780	**12**	480	**17**	180
3	1020	**8**	720	**13**	420	**18**	120
4	960	**9**	660	**14**	360	**19**	60
5	900	**10**	600	**15**	300	**20**	0

C. Serial Dilution of Standard

1. Label 8 polypropylene tubes or PCR stripwells as S1, S2, S3, S4, S5, S6, S7, and S8.
2. Transfer the reconstituted Standard into the tube labeled “S8”.
3. Add the appropriate amount of the Sample Diluent into the labeled tubes according to the table below (this will be sufficient for duplicate standard curves):

Standard	Vol of Sample Diluent	Vol of Std
S7	75 µL	25 µL of S8
S6	75 µL	25 µL of S7
S5	75 µL	25 µL of S6
S4	75 µL	25 µL of S5
S3	75 µL	25 µL of S4
S2	75 µL	25 µL of S3
S1	75 µL	25 µL of S2

4. Prepare working Standards at 1:4. Serial dilute the appropriate amount of Standard into each of the labeled tubes with Sample Diluent.
5. Vortex at a medium setting and ensure that each Standard is thoroughly mixed.

D. Sample Preparation – Note: Do not dilute controls

1. ***Optional*:** Samples containing active virus can undergo heat inactivation by placing samples at 56°C for 30 minutes prior to dilution.
2. Dilute samples in Sample Diluent 1:2000. A suggested dilution is outlined in table below

Sample Dilution	Volume of Sample		Volume of Sample Diluent
1:2000	(a) Prepare 1:10	5 µL	45 µL
	(b)Prepare 1:20	5 µL of (a)	95 µL
	Prepare 1:10	10ul of (b)	90 µL

E. Assay Protocol

1. Add 50 µL of 1X Assay Buffer to all wells of the plate.
2. Vortex the Beads at medium speed for 10-20 seconds. Add 10 µL of the Beads to all wells of the plate.
3. Wash plate 2X with 100ul of 1X Assay Buffer using either a magnetic bead separator, plate washer or equivalent. **(Do not resuspend in buffer)**
4. Add 30 µL of the Standard, Control or diluted Sample to the appropriate wells of the plate.
5. Cover plate. Place on plate shaker and incubate at medium-high speed for 10 seconds then decrease speed to medium and **incubate for 1 hour at room temperature in the dark.**

F. Prepare Detection

1. Vortex the 20X detection antibody stock at medium speed for 10-20 sec. Dilute **20X** stock to **1X** in 1X Assay Buffer. For a 96-well plate, dilute **210µL** of detection into **3990µL** of 1X Assay Buffer.

G. Wash Plate and Add Detection

1. Wash the plate 3X with 100µl of 1X Assay Buffer using either a magnetic bead separator, plate washer or equivalent. (Do not resuspend in buffer)
2. Add 40 µl of detection to each well.
3. Cover plate. Place on plate shaker and incubate at medium-high for 10 seconds then decrease speed to medium and **incubate for 30min at room temperature in the dark**.

H. Wash Plate

1. Wash the plate 3X with 100µl of 1X Assay Buffer using either a magnetic bead separator, plate washer or equivalent.
2. Resuspend in 100 µl of 1X Assay Buffer.

I. Mix Plate

1. Place plate on plate shaker and mix at medium speed for 10 minutes.

J. Read Plate

1. Read the Plate on the Luminex instrument.

Conditions of Sale

FOR IN VITRO RESEARCH USE ONLY. DO NOT USE FOR DIAGNOSTIC PROCEDURES. DO NOT USE IN HUMANS OR IN ANIMALS. USE UNIVERSAL PRECAUTIONS. *SERUM SAMPLES WERE TESTED BY PCR AND SHOWN BE NON REACTIVE FOR VIRSUES.