EUROIMMUN Anti-Measles & Rubella ELISA (IgG) Test Instructions

1 SPECIMEN AND REAGENTS PREPARATION

1. Remove specimens, controls, and reagents from the fridge and allow to reach room temperature before starting the assay
    a. Specimens
    b. Kit Reagents
    c. Positive and Negative Controls
2. Vortex specimens and controls
3. Dilute test samples 1:101 with the sample buffer, being sure to switch tips between each sample (ie: add **10 µl of plasma to 1000 µl of sample buffer**). Use a p1000 multichannel pipetter for sample buffer. Use p20 to add undliluted plasma. When adding plasma, mix well by repeatedly plunging the pipetter up and down at least 10 times. Do not aerosolize.
4. The four kit calibrators are ready-for-use—DO NOT DILUTE.
5. Prepare plate map by organizing specimen IDs on excel printout.
6. Dilute the 10X washing buffer concentrate with distilled water following the calculations on the Euroimmun worksheet. (e.g: if running 4 strips, add 20 ml washing buffer to 180 ml distilled water) *Buffer can be stored for up to 28 days at 2-8°C. If using refrigerated buffer, bring to room temperature before proceeding.*

2 SPECIMEN TEST PROCEDURE

1. Set up the Microplate with the correct number of individual strips to match the number of specimens and control as set out in the plate map
2. Using a new tip for each well, mix diluted plasma specimens well by repeatedly plunging the pipetter up and down at least 10 times. Add **100 µl** of the diluted plasmim into the designated well as indicated on the microplate map. Discard the pipette tip after each well. A multichannel pipette can be used.
3. Add **100 µl** of calibrators, positive control (blue) and negative control (green), and internal controls into designated individual microplate wells, as indicated on microplate map.
4. Incubate for **30 minutes** at room temperature. Prepare plate washer during incubation.
5. Wash and soak the wells 3 times with 300 µl working strength wash buffer using the Euroimmun wash program
6. Thoroughly dispose of all liquid from the microplate by tapping it on absorbent paper with open side down to remove all residual wash buffer
7. Add **100 µl** of enzyme conjugate (anti-human IgG) into each of the microplate wells
8. Incubate for **30 minutes** at room temperature. Enter data in Excel file during incubation.
9. Wash and soak the wells 3 times with 300 µl working strength wash buffer using the Euroimmun wash program
10. Add **100 µl** of chromogen substrate solution into each of the microplate wells. Substrate is sensitive to light. Do not open substrate until immediately before using it.
11. Incubate for **15 minutes** at room temperature in a drawer to protect from direct light. Prepare plate reader and computer during incubation.
12. Take the plate out of the drawer,
13. Add **100 µl** of stop solution into each well [in the same order as the chromogen substrate was added]
14. Slightly shake the microplate to ensure the homogeneous distribution of the solution (without individual wells spilling into each other) and read the microplate within 15 - 30 minutes at 450nm and within reference of 620 and 650 nm. Be sure Gen5 program is using 'delta' reading.