



Brief Instruction

Assay Total Time: ca. 3 hours

Preparations:

- 1) Allow microwell plate, reagents and patient sera to come to room temperature
- 2) Shake sample buffer well !
- 3) Dissolve 75 mL (1 bottle) washing buffer concentrate in 5 L aqua dest.

Assay Procedure:

- 1) - **pipet 100 μ L** calibrators (1,2,3,4), controls into intended wells and
- 2) - **pipet 20 μ L** patient samples into intended wells.
- **pipet 80 μ L** sample buffer to patient samples. Shake plate
- 3) Incubation 1: cover plate with lid and incubate **1 hour** at room temperature
- 4) Wash plate 5 times with each 400 μ L (automated washer in overflow mode)
- 5) - **pipet 100 μ L** conjugate per well
- 6) Incubation 2: cover plate with lid and incubate **1 hour** at room temperature
- 7) Wash plate 5 times with each 400 μ L (automated washer in overflow mode)
- 8) - **pipet 100 μ L** substrate solution per well
- 9) Incubation 3: cover plate with lid and incubate 10 minutes at room temperature under exclusion of light
- 10) - **pipet 100 μ L** stopping solution per well
- 11) Measurement of plate at 450 nm (620 nm reference wavelength)
- 12) Data analysis supported by computer software