



Brief Instruction

Preparations:

- 1) Allow microwell plate, reagents and patient sera to come to room temperature / shake all components
- 2) Shake sample buffer well!
Dissolve patient sera with sample buffer (1:100).
This corresponds to 15 μ L serum in 1,5 mL sample buffer (for 25 determinations)
- 3) Dissolve 1 bottle of washing buffer concentrate in 5 liter aqua dest.
Amount is sufficient for 2 plates. Durability: 1 week at room temperature
- 4) Dissolve 1 tablet p-NPP in one bottle substrate buffer.
Amount is sufficient for 200 determinations (2 plates)

Assay Procedure:

- 1) Aspirate remaining supernatant of disc buffer from wells
- 2) **Pipet 50 μ L** calibrators, controls and patient serum into intended wells
- 3) Incubate **1 hour** at 37 - 40°C. Cover plate with lid
- 4) Wash plate 6 times with each 1000 μ L (automated washer in overflow mode)
- 5) **Pipet 50 μ L** conjugate per well
Incubate 1 hour at 37 - 40°C. Cover plate with lid
- 6) Wash plate 6 times with each 1000 μ L (automated washer in overflow mode)
- 7) Shake substrate solution well.
Pipet 100 μ L substrate solution per well and
Incubate 45 minutes at 37 - 40°C. Cover plate with lid
- 8) **Pipet 50 μ L** stopping solution per well und shake plate briefly
- 8) Plate measurement at 405 nm (620 nm reference wavelength)
- 9) Data analysis supported by computer software