



Brief Instruction

Preparations:

- 1) Allow microwell plate, reagents and patient sera to come to room temperature
- 2) Dissolve 1 bottle of washing buffer concentrate in 5 liter aqua dest.
- 3) Dissolve 1 tablet p-NPP in one bottle substrate buffer

Assay Procedure:

- 1) Equip corresponding well amount of the microtiter plate according to working protocol. Always fill stripes with empty wells!
- 2) - **pipet 20 μ L** calibrators, controls and patient serum, and
- add **200 μ L** conjugate. Mix well
- 3) Incubate **1 hour at** room temperature. Cover plate with lid
- 4) Wash plate 5 times with each 400 μ L (automated washer)
- 5) Repeat mixing of substrate solution
- pipet **200 μ L substrate** solution per well
- 6) Incubate **30 minutes at** room temperature. Cover plate with lid
- 7) - **pipet 50 μ l** stopping solution per well
- 8) Plate measurement at 405 nm (620 nm reference wavelength)
- 9) Data analysis supported by computer software