

AspirinWorks®
Test Kit

Product #: 12136
(96 well kit)

- **Reagent complete kit, convenient procedure**
- **96-well microplate format**
- **Single wash step**
- **Assay time – 2.5 hours**

Background

Aspirin, the foundation of antiplatelet therapy in cardiovascular medicine, is widely prescribed by physicians to prevent heart attack and stroke. It is estimated that more than 150,000 heart attacks each year could be prevented by the appropriate use of aspirin therapy.

Recently, physicians discovered that a significant number of individuals taking dosages of aspirin considered therapeutic were experiencing vascular thrombotic events including acute coronary syndromes, transient ischemic attacks, strokes and peripheral vascular events. In addition, clinical researchers, utilizing a variety of laboratory tests, discovered that some patients have a reduced or minimal response to aspirin administration.

The AspirinWorks® Test Kit is a quantitative enzyme-linked immunoassay (ELISA) to determine levels of 11-dehydro thromboxane B2 (11dhTxB2) in human urine, which aids in the determination of platelet response to aspirin ingestion.

Principle and Procedure

The AspirinWorks® Test Kit measures urinary 11-dehydro thromboxane B2 (11dhTxB2) and is performed as a competitive ELISA. Diluted samples, purified 11dhTxB2 conjugated to alkaline phosphatase (AP), and purified mouse monoclonal antibody directed to 11dhTxB2 are combined and incubated in microwells coated with a polyclonal anti-mouse antibody. Incubation allows the endogenous 11dhTxB2 present in the samples to compete with the purified AP-conjugated 11dhTxB2 for binding to the mouse monoclonal anti-11dhTxB2 antibody. The monoclonal antibody then binds to the polyclonal anti-mouse antibody coated on the microtiter plate. The complex formed on the plate is composed of monoclonal antibody and endogenous or AP-conjugated 11dhTxB2. After the removal of unbound complexes by washing, the bound AP-11dhTxB2 conjugate is assayed by the addition of paranitrophenylphosphate (pNPP) chromogenic substrate. Color develops in the wells at an intensity inversely proportional to the sample urine concentration of 11dhTxB2, and is read on a spectrophotometer at 405nm. Results (pg/mL) are calculated against a reference curve prepared from the Reference Solution provided in the kit. Final results are reported as pg 11dhTxB2 per mg creatinine to normalize results for urine concentration.

Clinical Performance

AspirinWorks® results are presented as positive or negative, based on a cutoff of 1500 pg 11-dehydro thromboxane B2 per mg urinary creatinine. The detection range for 11-dehydro thromboxane B2 in the test is 300-4000 pg/mL urine. For greatest accuracy, samples that generate values greater than 4000 pg/mL should be retested at an appropriate dilution. The analyte concentration reported should be normalized by dividing the measured 11 dhTxB2 by the concentration of urine creatinine as measured by a separate assay.

The test kit was evaluated for intra-assay (72 replicates per sample within 1 kit lot) and inter-assay (216 replicates per sample over 3 kit lots) precision using the kit urine controls. Results are summarized in the following table:

Control	Overall Mean 11-dehydro thromboxane B2 Concentration	Intra-assay mean CV%	Inter-assay mean CV%
1	548 pg/mL	10%	13%
2	915 pg/mL	5%	8%
3	1765 pg/mL	4%	5%