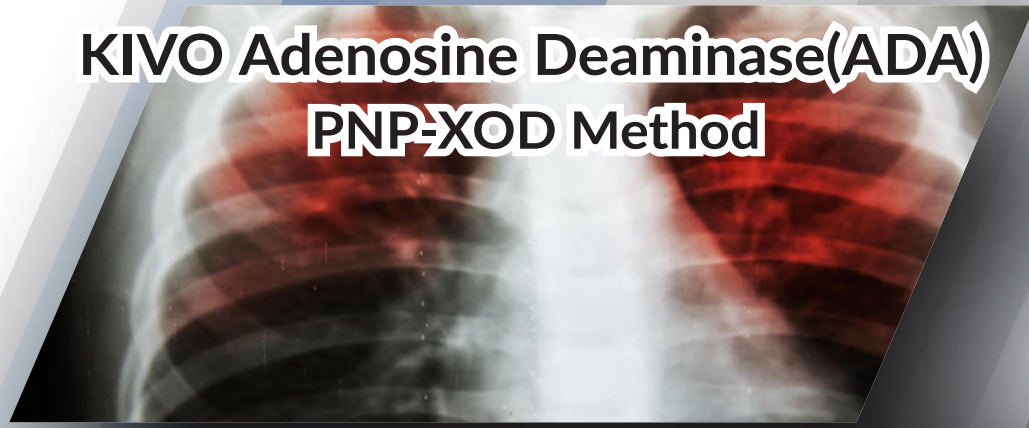




KIVO Adenosine Deaminase(ADA) PNP-XOD Method



Features and Advantages

- For the determination of Pulmonary and Extra Pulmonary Tuberculosis
- High Specificity: 98% (No febrile interference)
- Liquid Stable, ready to use two liquid reagents
- Lyophilized Calibrator provided.
- Low and High controls are available (Optional)
- 11 Minutes two step fixed time procedure.
(3 Minutes First Incubation + 8 Minutes Second Incubation)
- Fixed Time Kinetic reaction time 480 sec
(300 Sec Delay+ 180 Sec Measuring).
- High Linearity: 250 IU/L.
- Measuring Wavelength 546 nm.
- Available for multi analyzer platforms



KIVO Adenosine Deaminase(ADA) PNP-XOD Method

Assay Method



The ADA assay consists of four steps:

The ADA assay is based on the enzymatic deamination of adenosine to inosine which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H₂O₂) by xanthine oxidase (XOD). H₂O₂ is further reacted with N-Ethyl-N-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generate quinone dye which is monitored in a kinetic manner.

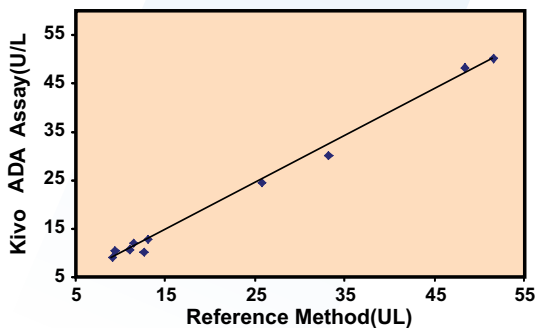
Performance

Method Comparison

To demonstrate accuracy, the High-Q Adenosine Deaminase Enzymatic Assay was tested with individual serum samples with comparison to results obtained by an accredited reference clinical laboratory using their analyte specific reagents based upon the reference method for ADA activity in serum.

The individual patient serum or plasma samples used for this study were from a certified commercial source. A small sample of ten (10) patient samples ranging from 13 – 48 U/L were tested which gave a correlation coefficient of 0.966. This study yielded a linear regression equation of $y = 0.9662x - 0.02$ U/L.

**Kivo ADA
Method Comparison**



Precision

The precision of the Kivo Adenosine Deaminase Enzymatic Assay was evaluated according to a modified Clinical and Laboratory Standards Institute (formerly NCCLS) EP5-A protocol. In the study, two specimens containing 11.0 ± 2.75 and 30.0 ± 5.4 U/L Adenosine Deaminase were tested with 2 runs per day with duplicates over 15 working days.

Assay Specifications:

Method	Enzymatic (Colorimetric / Kinetic)
Sample Type & Volume	• Serum • Plasma - Li-heparin Sample Volume 5 µL
Method Comparison	N = 15 y-intercept = -0.4219 Slope = 1.0688 R ² = 0.9894

Within-run Precision	Level 1	Level 2
Mean (U/L)	11.11	30.74
SD	0.16	0.45
CV%	1.47	1.45

Between-Run Precision	Level 1	Level 2
Mean (U/L)	9.63	29.62
SD	0.47	0.59
CV%	4.90	2.00

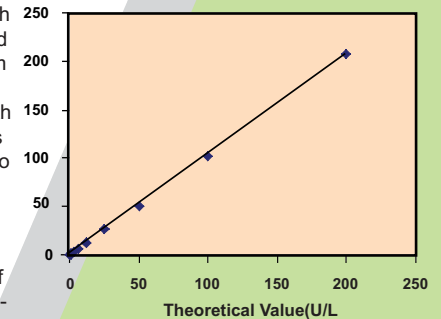
Analytical Sensitivity

To demonstrate the limit of detection (LOD) of Kivo Adenosine Deaminase Enzymatic Assay, Adenosine Deaminase zero calibrator was tested on 12 replicates on Cobas Mira. The LOD is defined as mean + 3SD. Based on these studies the LOD = $0.003 + 0.03 = 0.033$ U/L Adenosine Deaminase.

Linearity

Ten levels of samples with ADA activity were prepared by serially diluting a serum control containing 250 U/L Adenosine Deaminase with distilled H₂O. Based on this study the assay is linear to 200 U/L.

ADA Assay Linearity



Interference

To determine the level of interference from the substances normally present in the serum, Kivo Adenosine Deaminase Enzymatic Assay was evaluated by running three (3) replicates each of a control sample in the absence and presence of various potential interference substances at indicated concentrations. Assay is not affected by interfering substances such as serum bilirubin up to 30 mg/dL, hemoglobin up to 500 mg/dL, triglycerides up to 500 mg/dL, ascorbic acid up to 20 mg/dL, and ammonia up to 800 µmol.

Interfering substances	Interfering substance concentration	Concentration of ADA (U/L)	Nonspiking (control)	% Interference
Ammonia	800 µmol	22.38 ± 0.10	22.76 ± 0.14	1.6
Ascorbic Acid	4.0 mg/dL	8.70 ± 0.17	9.20 ± 0.21	5.4
Bilirubin	30 mg/dL	41.0 ± 0.18	41.15 ± 0.19	2.6
Hemoglobin	200 mg/dL	123.0 ± 0.36	117.9 ± 0.16	4.2
Triglycerides	500 mg/dL	17.53 ± 0.20	18.05 ± 0.26	2.9

Assay Specifications

Linearity	0 to 250 U/L
LOD	0.0333 U/L
Calibration Levels	1-Point Calibration
Reagent On-Board Stability	Opened: Four weeks when stored at 2-8°C

