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Isoniazid ELISA Kit

10/19

(Catalog # E4765-100; 96 assays, Store kit at -20°C)

I. Introduction:

Isoniazid, also known as isonicotinylhydrazide (INH), is a key drug used for the treatment of the deadly infectious disease tuberculosis (TB). TB is caused by a bacterium called Mycobacterium tuberculosis and is one of the most fatal diseases in the world. According to the Global tuberculosis report 2018, TB kills over 4,000 people each day. Currently INH is a first-line medication used to treat TB but it is often used along with other antibiotics such as rifampicin, pyrazinamide and/or ethambutol because of the raise of multidrug-resistant microorganisms. INH is a prodrug and must be first activated by the enzyme called KatG in Mycobacterium tuberculosis. Then the activated INH can interact with the cofactor NADH to form an adduct which binds tightly to the bacterial InhA enzyme to block cell wall synthesis. Some mycobacteria have evolved to mutate KatG to develop INH resistance and it has become a public health threat now. INH has some side effects such as numbness, higher liver enzymes in blood and liver inflammation and therefore it is important to monitor the INH concentrations in TB patients. The traditional techniques/instruments (HPLC or GC-MS) for detecting INH are expensive, laborious, and time-consuming. On the other hand, immunoassay techniques, such as ELISA, are commonly preferred as simple, reliable and rapid methods. BioVision's INH ELISA kit is a competitive-based ELISA that can quickly and reliably determine a broad range of INH concentrations in human serum samples. It can detect INH (0.2 – 45 µg/ml) within 90 minutes.

II. Applications:

In vitro quantitative determination of INH
Detection Range: 0.2-45 µg/ml
Sensitivity: 0.1 µg/ml

III. Sample Type:

Serum

IV. Kit Contents:

Components	E4765-100	Cap Code	Part Number
ELISA Microplate	8 X 12 Strips	--	E4765-100-1
INH Standard	2 vials	Yellow	E4765-100-2
HRP Conjugate Stock	25 µl	Blue	E4765-100-3
Antibody	7 ml	NM/Red	E4765-100-4
TMB substrate	12 ml	Amber	E4765-100-5
Stop Solution	10 ml	NM/Blue	E4765-100-6
Wash Buffer (10X)	50 ml	NM	E4765-100-7
Sample Diluent	20 ml	NM	E4765-100-8
Serum Solution	2 ml	Brown	E4765-100-9
Conjugate Buffer	7.5 ml	NM/Green	E4765-100-10
Plate Sealers	4	--	E4765-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Clean eppendorf tubes for preparing standards and sample dilutions

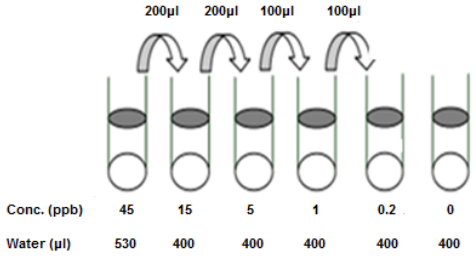
VI. Storage and Handling:

The entire kit may be stored at -20°C for up to 12 months from the date of shipment. Opened kit is stable for 2 months at 4°C.

VII. Reagent and Standard Preparation:

Bring all reagents to room temperature before use. Before using the kit, spin tubes and bring down all components to the bottom of tubes.

- **Antibody, TMB Substrate, Stop Solution, Sample Diluent, Serum Solution and Conjugate Buffer:** Ready to be used. After use, store them at 4°C.
- **HRP Conjugate Stock**: Spin briefly before opening the tube. Pipet 20 µl of HRP Conjugate Stock into Conjugate Buffer (7.5 ml) bottle to prepare conjugate working solution. Vortex the bottle for a minute. The conjugate working solution is stable at 4°C for 2 months.
- **Wash Buffer (10X)**: Bring bottle to room temperature. If crystals are present, warm up to room temperature and mix gently until the crystals are completely dissolved. Prepare 100 ml of 1X Wash Buffer by diluting 10 ml of Wash Buffer (10X) with 90 ml deionized water. The 1X solution can be stored at 4°C for one month.
- **INH Standards**: Reconstitute the INH standard by adding 530 µl of water to prepare 45 µg/ml standard (S5). Allow the solution to sit at room temperature for 10 minutes, then gently vortex to mix completely. Perform 3-fold dilution from S5 (e.g. mix 200 µl standard with 400 µl of water) to prepare standards S4 and S3 sequentially. Then perform 5-fold serial dilutions from S3 (e.g. mix 100 µl standard with 400 µl of water) to prepare standards S2 to S1 sequentially. S0 is water only. The standards are stable at -20°C for up to 3 weeks.



Standards	S0	S1	S2	S3	S4	S5
Concentrations (µg/ml)	0	0.2	1	5	15	45

VIII. Sample Preparation:

- **Serum**

1. Add 20 µl of Serum Solution into 180 µl of serum in an Eppendorf tube and vortex well. Incubate samples at 37°C for 45 min.
2. After the first incubation, incubate samples at 85-90°C for 10 min.
3. After 10 min, dilute the serum sample 20-fold using the Sample Diluent (For example, mix 10 µl of serum with 190 µl of Sample Diluent.)
4. Use 50 µl per well for the assay.

IX. INH ELISA Assay Protocol:

Notes: We recommend that all standards and samples are run in duplicate. A Standard curve must be run each time an assay is performed.

1. Prepare all reagents, standards and samples as sections VII and VIII specify respectively.
2. Add 50 µl of **Standards or Samples** per well. Then add 50 µl of **conjugate working solution** and 50 µl of **Antibody** to the above wells.
3. Cover the microtiter plate with plate sealer and mix well. Incubate the plate at room temperature (25°C) for 45 min.
4. Aspirate all reagents and wash each well 5 times: add 250 µl of **1X Wash Buffer** and incubate for 30 seconds. Remove 1X Wash buffer completely before the next wash. (Complete removal of wash buffer is essential for accurate results.) Repeat the wash step 4 more times.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate to ensure complete mixing.
6. Check the OD at 650 nm for the well containing no INH (S0). When its reading is approximately 0.8 (usually between 5-30 min after adding the TMB Substrate), add 50 µl of **Stop Solution** and gently tap the plate to ensure thorough mixing.
7. Measure the OD at 450 nm.

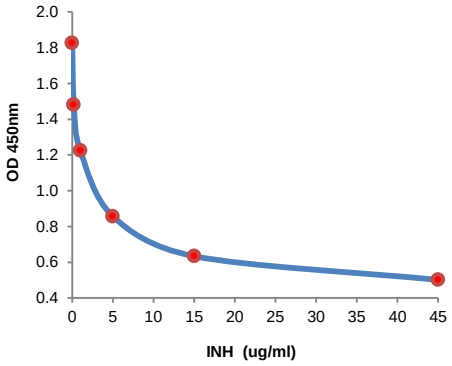
X. Calculation:

The mean values of relative absorbance are divided by the absorbance value of the zero-standard (A_0) and multiplied by 100%. The zero-standard is set to 100% and the relative absorbance of the standards and samples (A) are expressed as percentages.

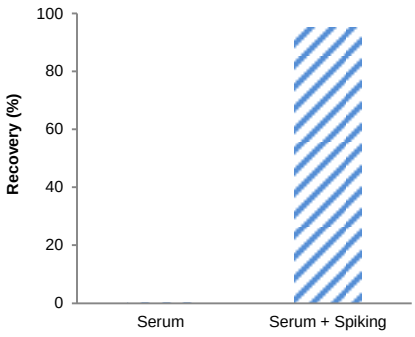
Relative Absorbance (%) = A/A_0 X 100%
A: The average absorbance of the standards or samples
A_0 : The average absorbance of the zero standard

The Standard Curve is done by plotting the relative absorbance of the standards vs. INH concentrations. The concentration of INH of each sample, which can be read from the calibration curve, is multiplied by the corresponding dilution factor.

A.



B.



Figures. **A.** INH standard curve (*This standard curve is for demonstration only. A standard curve must be run with each assay*). **B.** Spike recovery experiment: Human serum sample was assayed with and without INH spike (4 µg/ml) and showed >90% recovery.

XI. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315-100)
Folic Acid ELISA Kit (Cat. No. E4523-100)
Caffeine Acid ELISA Kit (Cat. No. E4558-100)
His-Tag Protein ELISA Kit (Cat. No. E4550-100)
DYKDDDDK-Tag Protein ELISA Kit (Cat. No.E4700-100)
Ampicillin ELISA Kit (Cat. No. E4350-100)
Quinolone ELISA Kit (Cat. No. E4530-100)
Vancomycin ELISA Kit (Cat. No. E4605-100)
GST Tag ELISA Kit (Cat. No. E4690-100)
Bisphenol A ELISA Kit (Cat. No. E4722-100)

FOR RESEARCH USE ONLY! Not to be used on humans.