Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Trimethoprim ELISA Kit

03/21

(Catalog # E4906-100; 96 assays, Store kit at -20 °C)

I. Introduction:

Trimethoprim (TMP) belongs to the list of essential medicines of World Health Organization. It is an antibiotic used mainly in the treatment of bacterial infections of human bladder, urinary tract and middle ear. TMP may be used against any susceptible gram-negative, aerobic bacterial species including *E. coli, Enterobacter, Klebsiella, Providencia, Morganella, Citrobacter, Salmonella* and *Shigella.* It may be also used in combination with other drugs to prevent kidney infections, lung infections and certain types of chronic inflammatory or autoimmune diseases. TMP is a potent dihydrofolate reductase (DHFR) inhibitor. It binds to dihydrofolate reductase and blocks the thymidine synthesis pathway thereby interfering with the bacterial DNA synthesis. Even though TMP is a very safe drug, it has some side effects such as nausea, diarrhea, rash, itching etc. **BioVision's Trimethoprim ELISA Kit** is a competitive enzyme immunoassay to determine the TMP concentration in various biological samples such as serum and urine. In this assay, the TMP antigen is pre-coated on the 96-well plate. TMP in the sample and the TMP antigen on the plate compete for binding to the anti-TMP antibody. After the addition of the HRP conjugate that binds to the captured TMP antibody, TMB substrate is added and a color signal is developed, which is measured at 450 nm. The OD value of the sample is inversely proportional to the TMP concentration in the sample. The OD value is then compared to the TMP standard curve and the TMP concentration is obtained. The kit is simple, easy and reliable tool to determine the TMP concentration (1 - 82 ng/ml) in approximately 90 minutes. The limit of detection of the kit is 0.5 ng/ml.

II. Application:

- *In vitro* quantitative determination of TMP in various biological samples.

III. Sample Types:

Serum and urine

IV. Kit Contents:

Components	E4906-100	Cap Code	Part Number
Pre-coated 96 Well Strip Plate	8 X 12 Strips	--	E4906-100-1
TMP Standard	1 vial	Yellow	E4906-100-2
HRP Conjugate	40 µl	Blue	E4906-100-3
Mouse Anti-TMP Antibody	7 ml	NM/Red	E4906-100-4
TMB Substrate	10 ml	NM/Amber	E4906-100-5
Stop Solution	10 ml	NM/Blue	E4906-100-6
Wash Buffer (10X)	50 ml	NM	E4906-100-7
Sample Diluent	20 ml	NM	E4906-100-8
Standard Buffer	20 ml	WM	E4906-100-9
Conjugate Buffer	7.0 ml	NM/Green	E4906-100-10
Plate Sealers	4	--	E4906-100-11

V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 and 650 nm
- Precision pipettes with disposable tips
- Eppendorf tubes
- Absorbent paper

VI. Storage and Handling:

Store the kit 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:

Briefly centrifuge all the small vials prior to opening. Read the entire protocol before performing the assay. Bring all reagents to room temperature (RT) before use.

- **TMP Standard:** Reconstitute the vial in 1.0 ml of Standard Buffer to prepare the **TMP Standard Stock** solution. Allow the solution to sit at RT for 10 min. Vortex gently to mix the contents completely. Store at -20 ºC.
- **HRP Conjugate:** Spin briefly before opening the tube. Pipet 35 µl of HRP Conjugate into the Conjugate Buffer bottle to prepare the **HRP Conjugate working** solution. Vortex the bottle for a min. The HRP Conjugate working solution is stable at 4 °C for 2 months.
- **Mouse Anti-TMP Antibody, TMB Substrate, Stop Solution, Sample Diluent, Standard Buffer** and **Conjugate Buffer:** Ready to use. Store at 4 °C.
- **Wash Buffer (10X):** Bring the bottle to RT. If crystals are present, warm up to RT 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.

VIII. TMP Standard Preparation:

1. Prepare 1 ml of **82 ng/ml TMP Standard (S5)** by mixing 100 µl of **TMP Standard Stock** solution with 900 µl of Standard Buffer.

2. Perform 3-fold serial dilutions of the Standards starting with S5 (82 ng/ml) to prepare S4 to S1 sequentially (as shown below). For example, mix 0.2 ml S5 Standard (82 mg/ml) with 0.4 ml of Standard Buffer to prepare S4 Standard (27 ng/ml). Prepare S3, S2, S1 TMP Standards accordingly. **Note:** Diluted TMP Standards are stable for up to 3 weeks at -20 °C.

TMP Standard	S0	S1	S2	S3	S4	S5
Concentrations (ng/ml)	0	1	3	9	27	82

IX. Sample Preparation:

- **Urine**

1. Centrifuge 0.2 ml of urine at 10,000 x *g* and RT for 5 min to remove any precipitate. Transfer 150 µl of supernatant into a clean eppendorf tube.
2. Dilute the sample 10-fold with Sample Diluent (i.e. mix 20 µl sample with 180 µl of Sample Diluent).
3. Use 50 µl per well for the assay.

- **Serum**

1. Dilute the serum 10-fold with Sample Diluent (i.e. mix 20 µl of serum with 180 µl of Sample Diluent).
2. Use 50 µl per well for the assay.

X. Trimethoprim ELISA Assay Protocol:

Notes:

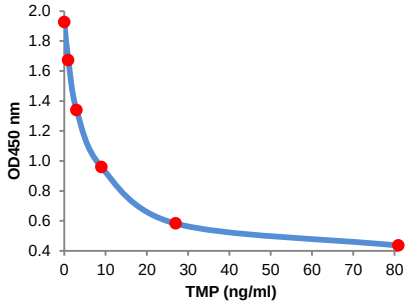
a. We recommended running all Standards and sample(s) in duplicates. A Standard Curve must be run with each assay
b. Prepare all reagents, Standards and sample(s) as instructed.
c. Use **1X Wash Buffer** for the assay.

1. Add 50 µl of **Standards or Samples** into appropriate wells.
2. Add 50 µl of **HRP Conjugate working solution** and 50 µl of **Mouse Anti-TMP Antibody** to the Standard and Sample wells.
3. Cover the microtiter plate with a plate sealer and mix well. Incubate the plate at RT (25 °C) for 75 min, protected from light.
4. Aspirate all reagents and wash buffer from each well 5 times. Wash by filling each well with 250 µl of **1X Wash Buffer** and incubating for 30 sec. Remove the 1X Wash Buffer completely before the next wash. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Clap the plate on absorbent filter papers or other absorbent materials. **Note:** Complete removal of Wash Buffer is essential for accurate results.
5. Add 100 µl of **TMB Substrate** to each well. Tap or shake the plate occasionally to ensure complete mixing.
6. Read the OD at 650 nm for the well containing no trimethoprim (S0) in kinetic mode. When the reading is approximately 0.8, add 50 µl of **Stop Solution** to all wells and gently tap the plate to ensure thorough mixing.
7. Measure the OD of the plate at 450 nm.

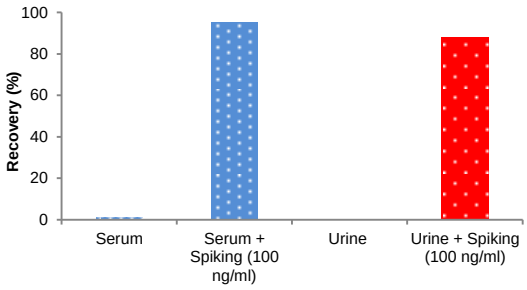
XI. Calculation:

Plot the TMP Standard Curve as relative OD 450 nm of each TMP Standard (Y) vs. the respective TMP concentrations (X). The concentration of TMP in the sample(s) is calculated from the TMP Standard Curve and reported as ng/ml. **Note:** If the sample(s) are diluted, multiply the dilution factor to the concentrations from interpolation.

A.



B.



Figures. **A.** Trimethoprim Standard Curve. *This Standard Curve is for demonstration only.* **B.** Spike recovery experiment using healthy human serum and urine samples spiked with TMP (100 ng/ml) respectively. Spike recovery was > 85.

XII. RELATED PRODUCTS:

Gentamicin (serum/urine) ELISA Kit (Cat. No. K4315)
Folic Acid ELISA Kit (Cat. No. E4523)
Caffeine Acid ELISA Kit (Cat. No. E4558)
His-Tag Protein ELISA Kit (Cat. No. E4550)
Isoniazid ELISA Kit (Cat. No. E4765)
Mycophenolic Acid ELISA (Cat. No. E4819)
Ampicillin ELISA Kit (Cat. No. E4350)
Quinolone ELISA Kit (Cat. No. E4530)
Vancomycin ELISA Kit (Cat. No. E4605)
GST Tag ELISA Kit (Cat. No. E4690)
Carnosine ELISA Kit (Cat. No. E4766)
Sulfadiazine ELISA Kit (Cat. No. E4904)

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