Data Sheet

Trimethoprim (TMP) ELISA Kit

Cat. #SG-4046

Size: 96 Wells

Sinogeneclon Co.,Ltd

Principle and Application

This kit adopts the method of competitive enzyme-linked immunoassay (ELISA) to detect Trimethoprim (TMP) in the sample such as tissue, serum, feed and urine. The kit is composed of Microtiter Plate coated with coupled antibodies, Enzyme-labeled antigen solution, standards and other supporting reagents. During the detection, with adding standards or samples, the TMP in the samples will compete with the coupled antibodies to combine with anti-TMP antigens. After adding HRP conjugates, take coloration with TMB substrates. Absorbance value of the samples is a negative correlation with TMP content. Lastly, by comparing the obtained absorbance values with the standard curve, we can calculate the TMP content in the sample.

Storage conditions

- The kit shall be stored at 2-8 ℃. Avoid freezing.
- Shelf Life: 12 months. The date of manufacture is presented in the label of the box.

Technique Data

- Kit Sensitivity: 0.015ppb (ng/mL)
- Reactive Mode: 37℃, 45min～15min
- Detection Limits:

Sample	**Detection Limits**
Feed	0.8ppb
Tissue (Fish, shrimp, meat, liver, kidney, etc.)	0.2ppb
Serum, urine, plasma	0.15ppb

- Cross-reaction Rate:

Drug name	**Cross-reaction Rate**
Trimethoprim	100%
Sulfapyridine	＜0.1%
Sulfanilamide	＜0.1%
Sulfadiazine	＜0.1%

Sulfafurazole	＜0.1%
Sulfathiazole	＜0.1%
Sulfamethazine	＜0.1%
Sulfadoxine	＜0.1%

- Sample Recover Rate:

Sample	**Recovery Rate**
Feed	85±10%
Tissue (Fish, shrimp, meat, liver, kidney, etc.)	85±15%
Serum, urine, plasma	85±10%

Composition of the Kit

Reagent	**Specification**
Microtiter Plate	8wells× 12strips
Standard: 0ppb, 0.015ppb, 0.045ppb, 0.135ppb, 0.405ppb, 1.215ppb (black cap)	1.0mL each
High Standard：100ppb (red cap)	1×1.0mL
Enzyme-labeled antigen solution (red cap)	1×5.5mL
Substrate Reagent A (white cap)	1×6mL
Substrate Reagent B (black cap)	1×6mL
Stop Solution (yellow cap)	1×6mL
Concentrated Wash Buffer (20×)(white cap)	1×40mL
Concentrated Reconstitution Buffer (2×) (yellow cap)	1×50mL
Instruction	1
Adhesive Membrane	1
Sealed bag	1

Materials Required but Not Supplied

- **Equipment:** microplate reader, printer, grinder (for homogenizing solid samples), nitrogen evaporator, vortex mixer (**for shake and mix**), centrifuge, graduated transfer pipette, and balance with a division value of 0.01 g, constant temperature device;
- **Micropipette:** single-channel (20-200μL and 100-1000μL), and multi-channel 300μL;

- **Reagents:** Anhydrous Methanol, n-Hexane, Concentrated Hydrochloric Acid (36% w/w), Sodium Hydroxide, Acetonitrile.

Experimental preparation

Restore all reagents and samples to room temperature (adjust to around 25°C) for more than 30 min before use. This is a crucial step to ensure there is no precipitation in the reagents.

Please note that the labware must be clean. Use disposable pipette tips to avoid contamination of interference results.

◆ **Solution preparation:**

Solution 1: Reconstitution Buffer

Dilute the Concentrated Reconstitution Buffer (2×) 2 times (Concentrated Reconstitution Buffer (2×) /Deionized water= 1:1) .The Reconstitution Buffer can be stored for one month at 4°C.

Solution 2: 0.1M Hydrochloric Acid Solution

Dilute 10 mL of concentrated hydrochloric acid with deionized water to a final volume of 1200 mL and mix thoroughly.

Solution 3: 0.1M Hydrochloric Acid Solution

Dissolve 4g of sodium hydroxide with deionized water to a final volume of 100mL.

Solution 4: Working Wash Buffer

Dilute the concentrated wash buffer (20**×)** by a factor of 20,

(Concentrated wash buffer/Deionized water= 1: 19)

◆ **Sample pretreatment steps:**

1. Feed treatment.

1) Weigh 2g ± 0.05g of ground sample into a 50mL centrifuge tube. Add 20mL of **0.1M hydrochloric acid solution (Solution 2)**. Shake for 15 minutes. Centrifuge at room temperature at 4000 r/min for 10 minutes.

2) Transfer 1mL of the supernatant to a 1.5mL centrifuge tube. Add 70μL of **1M sodium hydroxide solution (Solution 3)** to adjust the pH to 6-8. Mix well. (Adjust the amount of

1M sodium hydroxide solution depending on different feed samples if necessary). Centrifuge at room temperature at 4000 r/min for 10 minutes.

3) Transfer 0.5mL of the supernatant to another 1.5mL centrifuge tube. Add 0.5mL of the **Concentrated Reconstitution Buffer (2×)** and mix well.

4) Take 50μL for analysis.

Dilution times of the sample:20 Detection limits: 0.8ppb

2. Tissue (Fish, shrimp, meat, liver, kidney, etc) treatment.

1) Weigh 2g ± 0.05g of defatted homogenized tissue into a 50mL centrifuge tube. Add 6mL of anhydrous methanol and 2mL of n-hexane. Vortex at maximum speed for 5 minutes.

2) Centrifuge at room temperature at 4000 r/min for 10 minutes. Remove the upper n-hexane layer. Transfer 0.5mL of the lower clear liquid to a clean glass test tube (avoid touching the fat layer).

3) Evaporate to dryness under nitrogen or air at 50-60℃.

4) Add 400μL of the **Reconstitution Buffer (Solution 1)** and 500μL of n-hexane. Vortex at maximum speed for 1 minute.

5) Transfer to a 1.5mL centrifuge tube. Centrifuge at room temperature at 4000 r/min for 5 minutes. Remove the upper n-hexane layer. Transfer 50μL of the lower clear liquid for analysis.

Dilution times of the sample:5 Detection limits: 0.2ppb

3. Urine/serum/plasma treatment.

1) Take 0.5mL of the sample and centrifuge at room temperature at 4000 r/min for 5 minutes.

2) Transfer 50μL of the supernatant to a new container. Add 450μL of the **Reconstitution Buffer (Solution 1)** and mix well.

3) Take 50μL for analysis.

Dilution times of the sample:10 Detection limits: 0.15ppb

(If necessary, the volume of the **Reconstitution Buffer** can be increased to achieve a higher dilution factor.)

ELISA procedure

Place all reagents and samples to room temperature (adjust to around 25°C) for 30min. Gently shake the reagent bottles before use.

Take out the frame of the microplate along with the required number of wells. Then place the unused microplate wells into the sealed bag with the desiccant provided. Store the remaining kit in the refrigerator at 2-8°C.

Step 1: Number: Number the wells in sequence corresponding to the samples and standard, make 2-well parallel trials for each sample and standard, and record their locations.

Step 2: Incubation: Add 50μL of **standard or sample** into each numbered well, then add 50μL of **Enzyme-labeled antigen solution** per well. Finally, cover the Microtiter Plate with the adhesive membrane, shake gently by hand (or use a microplate shaker) for 5s and incubate for 45 min at 37℃ in the dark.

Step 3: Washing: Uncover the adhesive membrane carefully, discard liquid in the wells, pipette 350μL of **Working Wash Buffer (Solution 4)** to every well, let stand for 30 seconds then drain, repeat 5 times. Invert the plate and tap it against a thick absorbent paper (or lint-free cloth), with a soft towel placed underneath. (Bubbles that are not removed after tapping dry can be punctured with a clean pipette tip).

Step 4: Color: Add 50μL of **Substrate Reagent A** to each well. Then add 50μL of **Substrate Reagent B** per well. Shake gently by hand (or use a microplate shaker) for 5s, and allow to react for 15min at 37℃ in the dark. (The reaction can be extended appropriately if the blue color is too pale.)

Step 5: Stop the reaction: Pipette 50μL of **Stop Solution** to each well, and shake gently by hand (or use a microplate shaker). The reaction would be stopped.

Step 6: Calculate: Determine the Optical Density (OD value; absorbance value) at 450nm (Reference wavelength 630nm) with a microplate reader. Finish this step within 10min after stop the reaction.

Interpretation of result

- **Calculate the percentage of absorbance value**

Percentage of absorbance value(%)= $\frac{A}{A0} \times 100\%$

A—the average OD value of the sample or the standard;

A0—the average OD value of the 0ppb standard.

It is used to calculate the percentage absorbance of a standard or sample.

◆ **Draw the standard curve and calculate**

- Take absorbance percentage(A/A0) of standards as Y-axis and the corresponding log of standards concentration (ppb) as X-axis.
- Draw the standard semi-log curves with X-axis and Y-axis.
- Take absorbance percentage of samples substitute into standard curve, then can get the corresponding concentration from standard curve. **Last, the resulting concentration values multiplied by the corresponding dilution times is the actual concentration of TMP of samples.**

  If professional analysis software of the kit is used for calculation, it is more convenient for accurate and rapid analysis of a large number of samples.

Attention

- Before test, the reagents and samples should be balanced to room temperature (25℃). If below 25℃, it will lead to all the standard OD value on the low side.
- In washing process, dry wells may result in non-linear standard curves and undesirable reproducibility. Therefore, proceed to the next step immediately after washing.
- Please mix the contents within the wells uniformly and wash the plate thoroughly. The reproducibility is largely determined by consistency of washing step.
- During the incubation, cover microplates with adhesive membrane to avoid light.
- Do not use kits that are overdue. Do not mix reagents with those from other lots.
- Substrate Reagent A/B is colorless. If not, please discard.
- If absorbance value of 0ppb is below 0.5（A450nm< 0.5 ）, it means that the reagent may be metamorphic.
- Stop solution is corrosives. Please avoid contacting with skin.

- **As the OD values of the standard curve may vary according to the conditions of actual assay performance (e.g. operator, pipetting technique, washing technique or temperature effects), the operator should establish a standard curve for each test.**
- **For the mentioned sample, fast and efficient extraction technical support for the applicability if other sample need to be tested.**
- The kit is used for rapid screening of actual samples. If the test result is positive, the instrument method such as HPLC, LC/MS can be used for quantitative confirmation.