Streptomycin(SM) ELISA Kit

Catalog Number. CSB-E12087f

This immunoassay kit allows for the in vitro quantitative determination of Streptomycin(SM) concentrations in tissue, honey, milk, milk powder, eggs.

This package insert must be read in its entirety before using this product.

If You Have Problems

Technical Service Contact information

Phone: 86-27-87582341
Fax: 86-27-87196150
Email: tech@cusabio.com
Web: www.cusabio.com

In order to obtain higher efficiency service, please ready to supply the lot number of the kit to us (found on the outside of the box).

PRINCIPLE OF THE ASSAY

This assay employs the competitive inhibition enzyme immunoassay technique. The microtiter plate provided in this kit has been pre-coated with Streptomycin(SM). Standards or samples are added to the appropriate microtiter plate wells with an Streptomycin(SM) specific antibody and Horseradish Peroxidase (HRP) conjugated anti-antibody. The competitive inhibition reaction is launched between pre-coated Streptomycin(SM) and Streptomycin(SM) in standards or samples with the Streptomycin(SM) special antibody. A substrate solution is added to the wells and the color develops in opposite to the amount of Streptomycin(SM) in the standards or samples. The color development is stopped and the intensity of the color is measured.

DETECTION RANGE

0.1 ppb-8.1 ppb.

SENSITIVITY

The minimum detectable dose of Streptomycin(SM) is typically less than 0.1 ppb.

The sensitivity of this assay, or Lower Limit of Detection (LOD) was defined as the lowest concentration that could be differentiated from zero.

LIMIT OF DETECTION

Tissue	4 ppb
Honey	2 ppb
Milk, milk powder	5 ppb
Eggs	10 ppb

RECOVERY RATE

Tissue	85%±15%
Honey	85%±15%
Milk, milk powder	85%±15%
Eggs	85%±15%

CROSS-REACTION RATE

Streptomycin(SM)	100%
Dihydrostreptomycin	100%
Kamamycin	6.3%
Gentamicin	2.5%

PRECISION

Intra-assay Precision (Precision within an assay): CV% <10%
Three samples of known concentration were tested twenty times on one plate to assess.
Inter-assay Precision (Precision between assays): CV% <10%
Three samples of known concentration were tested in twenty assays to assess.

LIMITATIONS OF THE PROCEDURE

- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Any variation in operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- This assay is designed to eliminate interference by soluble receptors, binding proteins, and other factors present in biological samples. Until all factors have been tested in the Immunoassay, the possibility of interference cannot be excluded.

MATERIALS PROVIDED

Reagent	Quantity	Quantity
Assay plate	96T	48T
Standard	6 x 1 mL	6 x 0.5 mL
HRP-conjugate	1 x 11 mL	1 x 5.5 mL
Antibody	1 x 5.5 mL	1 x 2.7 mL
Substrate A	1 x 6 mL	1 x 3 mL
Substrate B	1 x 6 mL	1 x 3 mL
Stop Solution	1 x 6 mL	1 x 3 mL
Wash Buffer(20×)	1 x 40 mL	1 x 20 mL
Redissolving Solution(5×)	1 x 50 mL	1 x 25 mL
Adhesive Strip	4	4
Instruction Manual	1	1

STANDARD CONCENTRATION

Standard	S0	S1	S2	S3	S4	S5
Concentration (ppb)	0	0.1	0.3	0.9	2.7	8.1

STORAGE

Unopened kit	Store at 2-8°C. Do not use the kit beyond the expiration date
Opened kit	May be stored for up to 1 month at 2-8° C.

***Provided this is within the expiration date of the kit.**

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm.
- An incubator which can provide stable incubation conditions up to 25°C.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- Centrifuge, Vortex mixer.
- Analytical balance, 2 decimal place
- Single-channel micropipette(20 μL-200 μL、100 μL-1000 μL).
- 300 μL multichannel micropipette.
- Absorbent paper for blotting the microtiter plate.
- 100 mL and 500 mL graduated cylinders.
- Deionized or distilled water.
- Pipettes and pipette tips.
- Test tubes for dilution.
- NaOH.
- $Na_2HPO_4.12H_2O$
- $NaH_2PO_4.2H_2O$
- N-hexane
- Methanol
- Acetic Acid
- Concentrated Phosphoric Acid

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution. Wear eye, hand, face, and clothing protection when using this material.

Note:

- Kindly use graduated containers to prepare the reagent.
- Bring all reagents to room temperature (20-25°C) before use for 30 min.
- Only the disposable tips can be used for the experiments and the tips must be changed when used for different reagents.
- Distilled water is recommended to be used to make the preparation for reagents. Contaminated water or container for reagent preparation will influence the detection result.

REAGENT PREPARATION

- **0.05M PB buffer：** Take 12.9g **$Na_2HPO_4.12H_2O$** and 2.175g **$NaH_2PO_4.2H_2O$** into 1000 mL deionized or distilled water and shake well.
- **0.04M Phosphoric Acid(For honey use only):** Take 1 mL of **Concentrated Phosphoric Acid** into 360 mL deionized or distilled water and shake well.
- **1M NaOH(For honey use only):** Weigh 4g of **NaOH** into 100 mL deionized or distilled water and shake well.
- **1% Acetic Acid（for eggs use only)**:Take 1 mL of **Acetic Acid** into 99 mL deionized or distilled water and shake well.
- **70% Methanol（for eggs use only):**Take 700 mL of **Methanol** into 300 mL deionized or distilled water and shake well.
- **Redissolving Solution (1x)**: The **Redissolving Solution (5x)** is diluted with deionized water at 1:4 (eg: 1 mL **Redissolving Solution (5x)** + 4 mL deionized wate, shake well).
- **Wash Buffer**: If crystals have formed in the concentrate, warm up to room temperature and mix gently until the crystals have completely dissolved. Dilute 20 mL of **Wash Buffer (20x)** with deionized or distilled water to prepare 400 mL of **Wash Buffer (1x)**.

Note:

1. CUSABIO is only responsible for the kit itself, but not for the samples consumed during the assay. The user should calculate the possible amount of the samples used in the whole test. Please reserve sufficient samples in advance.
2. If the samples are not indicated in the manual, a preliminary experiment to determine the validity of the kit is necessary.
3. Please predict the concentration before assaying. If values for these are not within the range of the standard curve, users must determine the optimal sample dilutions for their particular experiments.

SAMPLE COLLECTION

A. Tissue

1. Weigh 2.00±0.05g of the homogenized sample (If high-fat, remove fat), put into centrifugal tube.
2. Add 8 mL of **0.05M PB buffer**,vortex for 5 min. Let it stand for 30 min at 56°C.
3. Centrifuge at above 4000 r/min for 10 min at room temperature.
4. Transfer 1 mL of supernatant and add 1 mL of **N-hexane**, shake well.
5. Centrifuge at above 4000 r/min for 5 min at room temperature.
6. Transfer 50 μL of under layer and 450 μL of **Redissolving Solution (1×)**, shake for 30s.
7. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 40

B. Honey

1. Weigh 2.00±0.05g of sample, put into centrifugal tube.
2. Add 4 mL of **0.04 M Phosphoric Acid**, vortex well until dissolve completely.
3. Add 450 μL of **1M NaOH** and adjust pH 7~9.

4. Centrifuge at above 4000 r/min for 5 min at room temperature until clear.
5. Transfer 50 μL of supernatant, then add 450 μL of **Redissolving Solution (1×)**, shake well.
6. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 20

C. Milk, milk powder

1. Weigh 2.00±0.05g of the homogenized sample, put into centrifugal tube.
2. Add 8 mL of **0.05M PB buffer**, vortex for 5 min.Let it stand for 30 min at 56°C.
3. Centrifuge at above 4000 r/min for 10 min at room temperature.
4. Transfer 50 μL of middle clear layer, then add 450 μL of **Redissolving Solution (1×)**, shake well.
5. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 50

D. Eggs

1. Weigh 1.00±0.05g of the homogenized sample, put into centrifugal tube.
2. Add 2 mL of **1% Acetic Acid**, vortex for 2 min. Then add 7 mL of **70% Methanol**, vortex for 2 min.
3. Centrifuge at 4000 r/min for 10 min at room temperature.
4. Transfer 100 μL of supernatant, add 900 μL of **Redissolving Solution (1×)**, shake well.
5. Take 50 μL of sample for further analysis.
   Dilution factor of the samples: 100

ASSAY PROCEDURE

Bring all reagents and samples to room temperature (20~25℃) before use. Centrifuge the sample again after thawing before the assay. It is recommended that all samples and standards be assayed in duplicate.

1. Prepare all reagents and samples as directed in the previous sections.
2. Determine the number of wells to be used and put any remaining wells and the desiccant back into the pouch and seal the ziploc, store unused wells at 2-8°C.
3. Add 50 µL of **Standard** or **Sample** per well. Standard and Samples need test in duplicate. Then add 50 µL of **Antibody** to each well. Mix well and then incubate for 30 min at 25°C.
4. Aspirate each well and wash, repeating the process 5 times. Wash by filling each well with 350 µL of **Wash Buffer (1x)** using a squirt bottle, multi-channel pipette, manifold dispenser, or autowasher, and let it stand for 30 seconds, complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining liquid by aspirating or decanting. Invert the plate and blot it against clean paper towels.
5. Add 100 µL of **HRP-conjugate** to each well. Mix well and then incubate for 30 min at 25°C.
6. Repeat the aspiration/wash process for 5 times as in step 4.
7. Add 50 µL of **Substrate A** and 50 µL of **Substrate B** to each well, mix well. Incubate for 15 min at 25°C. Protect from light.
8. Add 50 µL of **Stop Solution** to each well, gently tap the plate to ensure thorough mixing.
9. Determine the optical density of each well within 10 min, using a microplate reader set to 450 nm (Recommend to read the OD value at the dual-wavelength: 450/630 nm within 10 min).

Note:

1. The final experimental results will be closely related to validity of the products, operation skills of the end users and the experimental environments.
2. Samples or reagents addition: Please carefully add samples to wells and mix gently to avoid foaming. Do not touch the well wall as possible. For each step in the procedure, total dispensing time for addition of reagents or samples to the assay plate should not exceed 10 min. This will ensure equal elapsed time for each pipetting step, without interruption. Duplication of all standards and specimens, although not required, is recommended. To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
3. Incubation: To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary. Do not allow wells to sit uncovered for extended periods between incubation steps. Once reagents have been added to the well strips, DO NOT let the strips DRY at any time during the assay. Incubation time and temperature must be observed.
4. Washing: The wash procedure is critical. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Solution by aspirating or decanting and remove any drop of water and fingerprint on the bottom of the plate. Insufficient washing will result in poor precision and falsely elevated absorbance reading. When using an automated plate washer, adding a 15~30 second soak period following the addition of wash buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
5. Controlling of reaction time: Observe the change of color after adding Substrates (e.g. observation once every 10 min). Substrates should change from colorless or light blue to gradations of blue. If the color is too deep, add Stop Solution in advance to avoid excessively strong reaction which will result in inaccurate absorbance reading.
6. Substrates are easily contaminated. Substrates should remain colorless or light blue until added to the plate. Please protect it from light.
7. Stop Solution should be added to the plate in the same order as the Substrates. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrates.

CALCULATION OF RESULTS

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

$$\text{Absorbency value}(\%) = \frac{B}{B_0} \times 100\%$$

B ——the average absorbance value of the sample or standard

B_0 ——the average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Streptomycin(SM) standards solution (ppb) as x-axis.

The Streptomycin(SM) concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained. (The software offered together will facilitate the calculation process, it's suitable for accurate and fast analysis of large scale samples, please contact us)

Note:

- Discard the substrate with any color that indicates the degeneration of this solution; when the absorbance value of standard solution 0 of less than 0.5 indicates its degeneration.
- The optimum reaction temperature is 25℃, and too high or too low will result in the changes in the absorbance value and detecting sensitivity.