Sulfonamides ELISA Kit

Catalog No.: BCEK1003

Size: 96T

Range: 1 ppb- 27 ppb

Sensitivity: 1 ppb (μg / kg)

Detection time: 50 min

Storage and Expiration: Store at 2-8℃ for one year.

Application: For quantitative detection of sulfonamides in animal tissues and honey samples.

Principle of the Assay

This kit was based on an indirect competitive enzyme immunoassay. Its main principle is that the unlabeled antibody is incubated in the presence of its antigen (sample). These bound antibody/antigen complexes are then added to an antigen-coated well. The plate is washed, so unbound antibody is removed. (The more antigen in the sample, the more Ag-Ab complexes are formed and so there are less unbound antibodies available to bind to the antigen in the well, hence "competition".) The secondary antibody, specific to the primary antibody, is added. This second antibody is coupled to the enzyme. A substrate is added, and remaining enzymes elicit a chromogenic or fluorescent signal. The reaction is stopped to prevent eventual saturation of the signal.

Kit components

1. One 96-well sulfonamides microtiter plate (12 strips x 8 wells)
2. Standards: 0 ppb (blank / negative control), 1 ppb, 3 ppb, 9 ppb, 27 ppb (RTU), 1ml for each
3. Sample extraction Buffer(10 x):40 ml
4. Sulfonamides HRP conjugated reagent: 13 ml
5. Anti- sulfonamides antibody: 7 ml
6. Sample diluent buffer (2 x): 15 ml
7. Substrate A: 7 ml
8. Substrate B: 7 ml
9. Stop solution: 7 ml
10. Wash buffer (20 x): 40 ml

Material Required But Not Provided

1. Microtiter plate spectrophotometer (450 / 630 nm)
2. Homogenizer, centrifuge & centrifuge tubes (5 ml, 50ml)
3. Precise pipette and disposable pipette tips
4. ELISA shaker
5. N-Hexane
6. Deionized water

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Protocol

- **Preparation of sample and reagents**

1. Wash buffer

Dilute concentrated Wash buffer (Kit Component 10) 19-fold (1:19) with deionized water (i.e. add 10 ml of concentrated wash buffer into 190 ml of deionized water). Wash buffer work solution can be stored at 4℃ for one month.

2. Sample extraction Buffer

Dilute concentrated Sample extraction Buffer (Kit Component 3) 9-fold (1: 9) with deionized water (i.e. add 10 ml of concentrated Sample extraction Buffer into 90 ml of deionized water). Sample extraction Buffer work solution can be stored at 4℃ for one month.

3. Sample diluent buffer

Dilute concentrated Sample diluent buffer (Kit Component 6) (1:1) with deionized water (i.e. add 10 ml of concentrated sample diluent buffer into 10 ml of deionized water). Sample diluent buffer work solution can be stored at 4℃ for one month.

4. Sample

>> Tissues

A. Weigh out 1.0 g ± 0.05 g of the corresponding samples and place into a 50 ml of centrifugal tube, add 5 ml of 0.01 mol/L PBS and 2 ml of N-Hexane, shake for 2 min with a shaker.

B. Centrifuge for 5 min at 4,000 rpm.

C. Collect 100 µl of **the substratum** carefully to a new tube, add 100 µl of 1x Sample diluent buffer work solution, shake 30 seconds to mix thoroughly.

D. Pipette 50 µl for analysis. **Sample dilution factor: 10**

>> Honey

A. Weigh out 1.0 g ± 0.05 g of the corresponding samples and place into a 50 ml of centrifugal tube, add 5 ml of deionized water and 2 ml of N-Hexane, shake for 2 min with a shaker.

B. Centrifuge for 5 min at 4,000 rpm.

C. Collect 100 µl of **the substratum** carefully to a new tube, add 100 µl of 1x Sample diluent buffer work solution, shake 30 seconds to mix thoroughly.

D. Pipette 50 µl for analysis. **Sample dilution factor: 15**

- **Assay procedure**

1. Balance all reagents to room temperature (20 - 25 ℃) and shake each reagent before use.
2. Set standard, test sample and control (zero / blank) wells on the provided plate respectively, and then, record their positions. It is recommend to measure each standard and sample in duplicate.
3. Aliquot 50 µl of 0.2 ppb, 0.6 ppb, 1.8 ppb, 5.4 ppb, 16.2 ppb standard solutions into the standard wells. Add 0 ppb into the control well. And add 50 µl of prepared sample into sample wells.
4. Add 50 µl of anti- sulfonamides antibody (Kit component 5) to each well. Shake the plate by hand gently to mix thoroughly, cover the plate and incubate at 37℃ for 20 min in dark.
5. Discard the liquid of wells, and add 300 µl of washing buffer work solution into each well, soak for 20 seconds and discard the liquid again. Repeat this procedure two more times for a total of **THREE washes**. Clap the plate on the absorbent filter papers or other absorbent material to remove the liquid

from the wells after the last washing.

6. Add 100 µl of sulfonamides HRP conjugate reagent (Kit component 4) to the above wells, shake the plate by hand gently to mix thoroughly, cover the plate and incubate at 37℃ for 20 min in dark.

7. Discard the liquid of wells, and add 300 µl of washing buffer work solution into each well, soak for 20 seconds and discard the liquid again. Repeat this procedure two more times for a total of **THREE washes**. Clap the plate on the absorbent filter papers or other absorbent material to remove the liquid from the wells after the last washing.

8. Add 50 µl of Substrate A (Kit component 7) and then, add 50 µl of Substrate B (Kit component 8) into each well. Shake the plate by hand gently to mix thoroughly, cover the plate and incubate at 37°C for 10 min in dark.

9. Add 50 µl of Stop solution (Kit component 9) into each well and measure the absorbance at 450 nm immediately. (Double waves 450 / 630 nm are recommended.)

For calculation, (the relative $O.D._{450}$) = (the $O.D._{450}$ of each well) – (the $O.D._{450}$ of Zero well). The standard curve can be plotted as the relative $O.D._{450}$ of each standard solution (Y) vs. the respective concentration of the standard solution (X). A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ppb on a logarithmic curve. The concentration of sulfonamides in samples can be calculated on the curve by a professional analytical softeware.

Note: If the samples measured were diluted, multiply the dilution factor to the concentrations from interpolation to obtain the concentration before dilution.

Kit features and technical indexes

- Background value for purely negative samples: ＜ 2 ppb.
- Detection limit and accuracy for samples:

Samples	Detection limit	Recovery rate
Tissues	2 ppb (SD)	70 %-120 %
Honey	3 ppb (SD)	70 %-120 %

- **Cross-reactivity**

Corresponding antibiotics	Cross-reactivity (%)
Sulfadiazine	110 %
Sulfamonomethoxine, Sulfamethoxazole, Phthalylsulfathiazole, Sulfamethizole, Sulfapyridine	80 %
Sulfagan, Sulfamerazine, Catherine sulfanilamide, Sulfaxan	80 %
Sulfametoxydiazine	83 %
Sulfathiazole, Sulfamethazine	60 %

Troubleshooting

Problems	Possible reason	Recommended action
No Color Development or No Signals with Standards	Reagents were used in the wrong order or a step was skipped	Follow the protocol carefully and repeat the assay
	Reagents were expired or mixed with a	Make sure that the reagent used is kit-and

	different lot number	lot-specific
	Microplate is affected with damp	Make sure microplate is sealed well with desiccant
Low OD Readings	Reagents were expired or mixed with a different lot number	Verify the expiration dates and lot numbers
	Wash buffer was prepared incorrectly or washing time is too long	Make sure that it is prepared correctly, control washing time
	Incubation time was too short	Follow protocol, prolong incubation time for 5-10 min if possible
	Reagents were contaminated	Make sure to change tips for different solutions
	Reagents and plates were in cold condition	Make sure plates and reagents were balanced to room temperature. Keep the kit components out of the kit box for at least 1 hour before starting the assay.
	Reader was at wrong wavelength, or reader was malfunctioning	Make sure the wavelength is 450 nm for the assay and read the plate again. Verify reader calibration and lamp alignment
	Excessive kit stress has occurred	Check records to see how many times the kit has cycled from the refrigerator. Check to see if the kit was left at extreme temperatures for too long.
High Background or High OD Readings	Unsuitable deionized water was used to dilute the wash buffer	Change another deionized water to dilute
	RT is higher or incubation time is long	Make sure the time and temperature are suitable
	Insufficient washing or poor washer performance	Make sure to add at least 250 μl of wash buffer into each well per wash. Verify the washer system is working well
	Reader was malfunctioning or not blanked properly	Verify the reader's performance using a calibration plate and check the lamp alignment. Verify the blanking procedure, if applicable, and reblank
	Reagents were mixed or contaminated	Ensure to use the right reagent and avoid contamination.
High Intra-Plate Variance	Add standards, reagents or samples incoherently	Try to use a multichannel pipette to add reagents to multiple wells. Do not interrupt while adding standards, reagents and samples.
	Multichannel pipette was not functioning properly	Verify pipette calibration and check that tips are tight. Be sure all channels of the pipette draw and dispense equal volumes.

	Inconsistent washing or malfunctioning washer system	Check performance of the wash system. Repair the system if any ports drip or dispense/aspirate poorly.
High Inter-Plate Variance	Inconsistent incubation times occurred from plate to plate.	Time each plate separately to ensure consistent incubation times.
	Pipette was working improperly.	Check the pipette calibration. Verify that pipette tips are tight before use and all channels draw and dispense equal volumes.
	Reagents used were intermixed from different kit lots, or the kits were of different expiration dates.	Avoid mixing reagents from different lots, and ensure reagents added to all wells are from the same kit.
One or More of the Standard Curve Points Are Out of Range	Standards were added in wrong order or recorded in wrong position.	Follow the protocol and rerun the assay. Make sure the standards are applied and recorded correctly.
	Standards were contaminated or intermixed with other standards.	Use a new set of standards. Add standards to plate only in the order from low concentration to high concentration.
	Inconsistent washing or malfunctioning washer system	Perform washing consistently. Check performance of the wash system. Have the system repaired if any ports drip or dispense/aspirate poorly.
	Add standards, reagents or samples incoherently	Make sure all materials are set up and ready to use. Add standards to plate only in the order from low concentration to high concentration at undisrupted pace. Use a multichannel pipette to add reagents to multiple wells simultaneously.
	Multichannel pipette was not functioning properly.	Verify pipette calibration and check that tips are tight. Be sure all channels of the pipette draw and dispense equal volumes.

Precautions

1. Run the next procedure immediately after washing plate.
2. The Stop solution (Kit component 9) is 2 M sulfuric acid, particular care should be taken when operating.
3. If the absorbance value of 0 ppb is less than 0.5 units ($A_{450\ nm}$ < 0.5), the reagents may not work well.
4. Do not use the reagents after expiry and not interchange individual reagents between kits of different lot numbers.
5. The incubation time for color development (Step 8) is usually 15 min, if the blue color is very light, the incubation time can be prolonged to 20 min, vice versa.
6. Substrate B (Kit Component 7) may turn to watchet blue for long term storage, but its effect will NOT be influenced.

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