Technical specifications
Sensitivity: 1 ppb
Detection limit
Tissue.....1 ppb
Serum, Urine.....4 ppb
Milk.....20 ppb
Recovery rate
Tissue.....70 ±10%
Egg.....66 ±10%
Milk, honey, serum.....70 ±10%
Cross-reaction rate
Sulfadiazine (SD).....100%
Sulfamonomethoxine (SMM).....97.6%
Sulfamerazine (SM1).....12%
Sulfamethoxazole (SMZ).....100%
Sulfathiazole (ST).....195%
Phthalylsulfathiazole (PST).....73%
Sulfamethyltiadiazolum.....110%
Sulfapyridine.....48%
Sulfamethoxydiazine(SMD).....26.4%
Sulfafurazole(SIZ).....95.0%

This test kit is used to detect 5 Sulfonamides based on cross-reaction rate, its detection limit as follows:
SD — 1 ppb
SMM — 1 ppb
SMZ — 2 ppb
ST — 0.5 ppb
SM1 — 10 ppb
PST — 2 ppb
SMD — 10 ppb
Sulfamethyltiadiazolum — 1 ppb
Sulfapyridine — 2 ppb
SIZ — 1 ppb

Antibiotics ELISA kits available from ADI:

DE-100010	Clenbuterol ELISA kit, 96 tests (For Urine, Serum, Feed, Meat, Liver)
DE-100020	Ractopamine ELISA kit, (For Liver, Urine, Feed), 96 tests
DE-100030	Salbutamal ELISA kit, For Urine, Tissue, Feed, Animal Tissue, Aquatic, Honey, Intestine,, 96 tests
DE-100040	Chloramphenicol ELISA kit, 96 tests (For Animal Tissue, Aquatic, Honey, Intestine, Urine, Egg, Milk, Serum)
DE-100050	Florfenicol ELISA kit (For Animal Tissue, Aquatic, Honey), 96 tests
DE-100060	Nitrofuran (AMOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100070	Nitrofuran (AHD) ELISA kit, (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100075	Nitrofuran (SEM) ELISA kit (Honey, Fish, Shrimp, Chicken/Liver, Fish/Shrimp), 96 tests
DE-100080	Nitrofuran (AOZ) ELISA kit (For Fish, Shrimp, Honey, Chicken/Liver), 96 tests
DE-100090	Sulfonamides Residues (SAs) ELISA kit, (For Chicken/Liver, Pork/Liver, Honey/Egg, Serum/Urine, Milk), 96 tests

See Details at the web site or Contact ADI

Life Technologies™
Your Molecular & Cell Technology Partner

adi

Instruction Manual No. M-DE-100090

Sulfonamides (SAs) ELISA KIT

Cat. #DE-100090

For Qualitative and Quantitative Determination of SMM, SD, SM1, SMZ, ST, PST, Sulfamethyltiadiazolum, Sulfapyridine, SMD and SIZ in chicken liver, pork liver, serum, urine, milk, honey and egg.

India Contact:
Life Technologies (India) Pvt. Ltd.
306, Aggarwal City Mall, Road No. 44, Pitampura, Delhi – 110034, India
Mobile: +91-98105-21400, Tel: +91-11-42208000, 8111, 8222, Fax: +91-11-42208444
Email: customerservice@lifetechindia.com, www.atzlabs.com ; www.lifetechindia.com

Sulfonamides (SAs) ELISA KIT Cat. #DE-100090

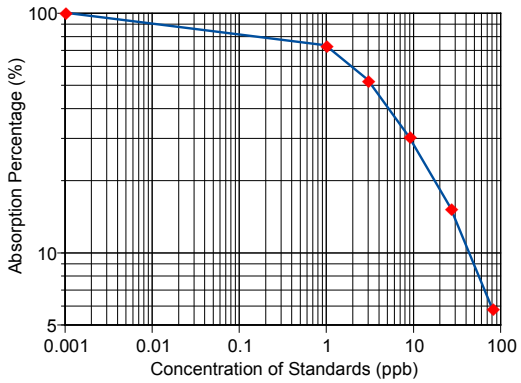
Kit Components, 96 tests	Cat #
Micro-well coated strip plate (12 strips with 8 removable wells each)	DE-100091
6x standard solution (1 ml each): 0 ppb, 1 ppb, 3 ppb, 9 ppb, 27 ppb, 81 ppb	DE-100092
Enzyme conjugate (7 mL)	DE-100093
Antibody working solution (10 mL)	DE-100094
Substrate A solution (7 mL)	DE-SSA
Substrate B solution (7 mL)	DE-SSB
Stop solution (7 mL)	DE-ST
20x concentrated washing buffer (40 mL)	DE-WB
2× concentrated redissolving solution (50 mL)	DE-SS2
Instruction Manual	M-DE-100090

INTRODUCTION

Sulfamonomethoxine (SMM), Sulfadiazine (SD), Sulfamerazine (SM1), Sulfamethoxazole (SMZ) and Sulfathiazole (ST), Phthalylsulfathiazole (PST) Sulfamethyltiadiazolum, Sulfapyridine, Sulfamethoxydiazine (SMD) and Sulfafurazole (SIZ) are sulfonamide based antibiotic. Sulfonamide is also known as sulfa drugs. It is used as an antimicrobial and plays a role of competitive inhibitor of the enzyme dihydropteroate synthetase. Sulfonamide is also present in some other medications that do not act as an antimicrobial; they include thiazide diuretics, loop diuretics sulfonylureas, some COX-2 inhibitors and acetazolamide. Sulfonamide is an organic sulfur compound containing the amides of sulfonic acids. Its molecular structure is similar to p-Aminobenzoic acid (PABA) which is needed in bacteria organisms as a substrate of the enzyme dihydropteroate synthetase for the synthesis of thetrahydrofolic acid (THF). PABA is not a vitamin but it is essential for the human body. In human body PABA is produce by E. coli in the colon, so human does not have the need to intake from food. Sulfa drugs will mainly work against bacteria which require PABA for their growth.

Sulfonamide drugs were the first antimicrobial drugs which opened the doors for many other antimicrobial drugs. Sulfa drugs played a major role during World War II. It saved lives of tens of thousands of patients including the son of the president Franklin Delano Roosevelt, Franklin Delano Roosevelt, Jr. and Winston Churchill. Sulfa drug powder was included in the first aid kit and it was recommended to be sprinkled on any open wound. Sulfonamide works better in a basic environment, the solubility of the compound is very low and sometimes it can form crystals in the kidneys.
The reaction of a sulfonyl chloride with ammonia or an amine will produce sulfonamides.

The uses of sulfonamides include urinary tract disorders, haemopoietic disorders, porphyria and hypersensitivity reactions. A strong allergic reaction



A typical assay Standard Curve (do not use this for calculating sample values)

CALCULATION OF RESULTS

There are two methods to judge the results: the first one is the rough judgment, while the second is the quantitative determination. Note that the OD value of the sample has a negative correlation with the SMM, SD, SM1, SMZ, ST, PST, Sulfamethyltiadiazolum, Sulfapyridine, SMD SIZ concentration.

Qualitative determination
The concentration range (ng/mL) can be obtained from comparing the average OD value of the sample with that of the standard solution. Assuming that the OD value of the sample Ⅰ is 0.211, and that of the sample Ⅱ is 0.785, the OD value of standard solutions is: 2.140 for 0 ppb, 1.560 for 1 ppb, 1.124 for 3 ppb, 0.650 for 9 ppb, 0.328 for 27 ppb ,0.125 for 81 ppb, accordingly to the concentration range of the sample Ⅰ is 27 to 81 ppb, and that of the sample Ⅱ is 3 to 9 ppb (multiplied by the corresponding dilution fold).

Quantitative determination
The mean values of the absorbance values is obtained for the average OD value (B) of the sample and the standard solution divided by the OD value (B0) of the first standard solution (0 standard) and subsequently multiplied by 100%, that is,

$$\text{Percentage of absorbance value} = \frac{B}{B_0} \times 100\%$$

B—the average OD value of the sample or the standard solution
B_0—the average OD value of the 0 ng/mL standard solution

Draw the standard curve with the absorption percentages of the standard solution and the semilogarithm values of the Sulfonamides standard solution (ng/mL) as Y- and X-axis, respectively. Read the corresponding concentration of the sample from the standard curve by incorporating its absorption percentage into the standard curve. The resulting value is subsequently multiplied by the corresponding dilution fold, finally obtaining the Sulfonamides (SMM, SD, SM1, SMZ, ST, PST, Sulfamethyltiadiazolum, Sulfapyridine, SMD SIZ) concentration in the sample. Using the professional analyzing software of this kit will be more convenient for the accurate and rapid analysis of a large amount of samples. (Please contact us for this software).

4. Numbering: number the micro-wells according to samples and standard solution; each sample and standard solution should be performed in duplicate; record their positions.
5. Add 20 µL of the sample or standard solution to separate duplicate wells, and add 50 µL of the enzyme conjugate, and then 80 µL of the antibody working solution into each well. Mix gently by shaking the plate manually, seal the microplate with the cover membrane, and incubate at 25℃ for 1 h.
6. Wash the microplate with the washing buffer at 250 µL/well for 4-5 times. Each time soak the well with the washing buffer for 10 s, flap to dry with absorbent paper (if there are the bubbles after flapping, cut them with the clean tips).
7. Coloration: add 50 µL of the substrate A solution and then 50 µL of the B solution into each well. Mix gently by shaking the plate manually, and incubate at 25 ℃ for 20-30 min at dark for coloration (See precaution 8).
8. Determination: add 50 µL of the stop solution into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (we recommend to read the OD value at the dual-wavelength 450/630 nm within 5 min).

NOTES:

1. The room temperature below 20 ℃ or the temperature of the reagents and the testing samples being not returned to the room temperature (20-25 ℃) will lead to a lower standard OD value.
2. Dryness of the microplate in the washing process will be accompanied by the situations including the non-linear standard curves and the undesirable reproducibility; So continue to next step immediately after washing.
3. Mix evenly, otherwise there will be the undesirable reproducibility.
4. The stop solution is the 2 M sulfuric acid solution, avoid contacting with the skin.
5. Do not use the kit exceeding its expiry date. The use of diluted or adulterated reagents from the kits will lead to the changes in the sensitivity and the detecting OD values. Do not exchange the reagents from the kits of different lot numbers to use.
6. Put the unused microplate into an auto-sealing bag to re-seal it. The standard substance and the colorless color former is light sensitive, and thus they cannot be directly exposed to the light.
7. Discard the coloration solution with any color that indicates the degeneration of this solution. The detecting value of the 0 standard solution of less than 0.5 (A_{450nm}< 0.5) indicates its degeneration.

Work Sheet of Typical Assay-Sulfonamides

(SMM, SD, SM1, SMZ, ST, PST, Sulfamethyltiadiazolum, Sulfapyridine, SMD SIZ)

Wells	Stds/samples		Mean $A_{450\ nm}$	Absorption Percentage
A1, A2	**Standard A**	0 ppb	2.143	100%
B1, B2	**Standard B**	1 ppb	1.560	72.80%
C1, C2	**Standard C**	3 ppb	1.124	52.45%
D1, D2	**Standard D**	9 ppb	0.650	30.33%
E1, E2	**Standard E**	27 ppb	0.328	15.31%
F1, F2	**Standard F**	81 ppb	0.125	5.83%

NOTE: These data are for demonstration purpose only. A complete standard curve must be run in every assay to determine sample values. Each laboratory should determine their own normal reference values.

might occur if taken in large amounts the two most serious are Stevens Johnson syndrome and Lyell syndrome. Adverse reactions when sulfa drugs are taken are very low, only 3% of the population will show symptoms.

Alpha Diagnostic Intl's Sulfonamides (SAs) ELISA kit is a highly sensitive competitive type assay for the measurement of SMM, SD, SM1, SMZ, ST, PST, Sulfamethyltiadiazolum, Sulfapyridine, SMD SIZ in chicken liver, pork liver, milk, serum, urine, honey, egg.

PRINCIPLE OF THE TEST

This test kit is based on the competitive enzyme immunoassay for the detection of Sulfonamides in the chicken, pork, milk, honey and egg, etc. The coupling antigens are pre-coated on the microwell stripes. The Sulfonamides in the sample and the coupling antigens pre-coated on the microwell stripes compete for the anti-Sulfonamides antibodies. After the addition of the enzyme conjugate, the TMB substrate is added for coloration. The optical density (OD) value of the sample has a negative correlation with the Sulfonamides in the sample. This value is compared to the standard curve and the Sulfonamides concentration is subsequently obtained.

MATERIALS AND EQUIPMENT REQUIRED

Equipments: microplate reader, printer, mixer or stomacher, oscillator, centrifuge, nitrogen-drying device, measuring pipettes and balance (a sensibility reciprocal of 0.01 g)
Micropipettors: single-channel 20 to 200 µL and 100 to 1000 µL, and multi-channel 250 µL.
Reagents: Acetonitrile (CH3CN), ethyl acetate, N-hexane, Na2HPO4·12H2O, NaH2PO4·2H2O, NaCl.

PRECAUTIONS AND SAFETY INSTRUCTIONS
The Sulfonamides (SAs) Kit is for research use only.
Stop Solution contains 1% sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.
MSDS for TMB, sulfuric acid, if not already on file, can be requested or obtained from the ADI website.

SAMPLE PRE-TREATMENT

Instructions
The following points must be dealt with before the pre-treatment of any kind of sample:
1. Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.
2. Before the experiment, each experimental equipment must be clean and should be re-cleaned if necessary, in order to avoid the contamination that interferes with the experimental results.

Solution preparation before sample pre-treatment
1. 2 M NaCl: dissolve 11.69 g NaCl in deionized water to 100 mL.
2. 0.2 M NaOH: dissolve 0.8 g NaOH in deionized water to 100mL.
3. 0.5 M HCL (for honey): dissolve 4.3 mL HCl (36%) in deionized water to 100 mL.

4. 0.02 M PB buffer: dissolve 2.58 g Na_2HPO_4 $12H_2O$ and 0.44 g NaH_2PO_4 $2H_2O$ in the deionized water to 500 mL (for high-detection-limit samples)
5. Acetonitrile (CH_3CN)-water solution: V_{CH3CN}/V_{H2O} = 84:16
6. The 2× concentrated redissolving solution is mixed with deionized water at 1:1 (1 mL 2× concentrated redissolving solution+1 mL deionized water), for the sample redissolving.

Samples preparation

High-detection-limit samples

a) Animal tissue meat, liver, shrimp, fish, egg.

1. Take the sample, homogenize at 10000 r/min for 1 min.
2. Weigh 3 ± 0.05 g of the homogenized sample, put into centrifugal tube, add 9 mL of the CH_3CN-water solution, shake properly for 10 min, centrifuge at above 4000 r /min at 15 ℃ for 10 min.
3. Transfer 4 mL of the supernatant into a new vessel, add 2 mL 2 M NaCl solution and 7 mL of ethyl acetate, shake for 5 min, and centrifuge at above 3000 r/min at room temperature for 5 min.
4. Transfer the supernatant into a new vessel, blow to dry with nitrogen completely by rotary evaporator at 50 ℃.
5. Add 1 mL of the diluted redissolving solution, shake for 1 min, add 1 mL N-hexane, mix for 2 min and centrifuge at 4000 r/min at room temperature for 5 min, remove the liquid (upper layer).
6. Take 20 μL of the lower for further analysis.

Fold of dilution of the sample: 1

It needs five fold dilution of the sample (1 mL sample+4 mL of the diluted redissolving solution) if the detection is based on the most residue (100 ppb) of national regulation.

Low-detection-limit samples

b) Animal tissues (meat, liver)

1. Weight 2.0 ± 0.05 g of the sample, add 10 mL 0.02 M PB buffer, shake upside down for 10 min, put into 37 ℃ constant temperature container for 30 min, centrifuge at above 5000 r/min at room temperature for 10 min.
2. Take 20 μL of the clear supernatant (upper layer) for further analysis.

Fold of dilution of the sample: 5 Detection limit: 5 ppb

c) Animal tissues (chicken, liver)

1. Take 2.0 ± 0.05 g of the sample, add 10 mL 0.02 M PB buffer and 5 mL N-hexane, shake upside down for 10 min, centrifuge at above 5000 r/min at 10 ℃ for 10 min.
2. Remove N-hexane phase (upper layer), take 100 μL of the lower, add 100 μL 0.02 M PB buffer, mix properly.
3. Take 20 μL for analysis

Fold of dilution of the sample: 10 detect limit :10 ppb

d) Serum

1. Place sample at room temperature for 30 minutes, centrifuge at above 4000 r/min at room temperature for 10 min, separate or filter serum.

2. Take 1 mL serum, add 3 mL 0.02M PB buffer, mix properly.
3. Take 20 μL for further analysis.

Fold of dilution of the sample: 4 detect limit :4 ppb

e) Honey

1. Put 1.0 ± 0.05 g honey into 50 mL centrifugal tube, add 1 mL 0.5 M HCl at 37℃for 30 min.
2. Add 2.5 mL 0.2 M NaOH (pH is approx 5), add 4 mL ethyl acetate, shake for 10 min, centrifuge at above 4000 r/min at room temperature (20-25 ℃) for 10 min.
3. Take 2 mL supernatant, blow to dry with nitrogen at 50 ℃, add 0.5 mL of the diluted redissolving solution, redissolve it.
4. Take 20 μL for further analysis

Fold of dilution of the sample: 1

f) Urine

1. And 3 mL 0.02 M PB buffer and 1 mL of the centrifuged clear sample, mix properly.
2. Take 20 μL for further analysis

Fold of dilution of the sample: 4 detect limit :4 ppb

g) Milk

1. Take 1 mL milk, add 0.02 M PB buffer, dilute at 1:20 (V/V) (20 μL milk + 380 μL 0.02 M PB buffer).
2. Take 20 μL for further analysis.

Fold of dilution of the sample:20 detect limit :20 ppb

STORAGE AND STABILITY

Storage: store at 2 to 8 ℃, not frozen.

Expiration date: 12 months; date of production is on box.

Instructions

1. Bring all reagents and micro-well strips to the room temperature (20-25℃).
2. Return all reagents to 2-8℃ immediately after use.
3. The reproducibility of the ELISA analysis, to a large degree, depends on the consistency of plate washing. The correct operation of plate washing is the key point in the procedures of ELISA.
4. For the incubation at constant temperatures, all the samples and reagents must avoid light exposure, and each microplate should be sealed by the cover membrane.

Operation Procedure

1. Take out the kit from the refrigerated environment. Take out all the necessary reagents from the kit and place at the room temperature (20-25℃) for at least 30 min. Note that each reagent must be shaken to mix evenly before use.
2. Take the required micro-well strips and plate frames. Re-sealed the unused microplate, stored at 2-8℃, not frozen.
3. Solution preparation: dilute 40 mL of the 20× concentrated washing buffer with the distilled or deionized water to 800 mL (or just to the required volume) for use.