

Ecologiena[®]

LAS ELISA KIT
(Microplate)

User's Guide



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LIMITED WARRANTY

Tokiwa Chemical Industries CO.,Ltd. (the Company, hereunder) warrants its products. (the Product, hereunder) to be manufactured in accordance with its specifications and free from defects in material. This warranty is expressly limited to the refund of the price of any defective Product or the replacement of any defective Product with new Product. This warranty applies only when the Buyer gives written notice to the Company within thirty (30) days after the receipt of the Product by the Buyer. In addition, this warranty applies under conditions of normal use, but does not apply to defects that result from intentional damage, negligence or unreasonable use.

THE COMPANY MAKES NO WARRANTIES, EITHER EXPRESS OR IMPLIED, EXCEPT AS PROVIDED HEREIN, INCLUDING WITHOUT LIMITATION THEREOF, WARRANTIES AS TO MARKETABILITY, MERCHANTABILITY, FITNESS FOR A PARTICULAR PURPOSE OR USE, OR NON-INFRINGEMENT OF ANY INTELLECTUAL PROPERTY RIGHTS. IN NO EVENT SHALL THE COMPANY BE LIABLE FOR ANY INDIRECT, INCIDENTAL, OR CONSEQUENTIAL DAMAGES OF ANY NATURE, OR LOSSES OR EXPENSES RESULTING FROM ANY DEFECTIVE PRODUCT OR THE USE OF ANY PRODUCT.

The design of the Product is under constant review and every effort is made to keep this guide up to date, the Company reserves the right to change specifications and equipment at any time without prior notice.

Kit Feature

- ✧ Linear Alkylbenzenesulfonate (LAS) monoclonal antibody binds exclusively with LAS and does not show cross-reaction with other chemicals of similar structures. A monoclonal antibody is uniform in quality, generating very little lot-to-lot variation.
- ✧ The quantitative analysis ranges from 0.02mg/L to 1mg/L (ppm).
- ✧ A simple filtration through glass filter is generally sufficient as a pretreatment before measurement. Solid phase extraction may be necessary if sample is required for concentration and/or clean-up.
- ✧ The LAS ELISA measurement is highly reproducible; the coefficient of variation (CV) is mostly under 10%.
- ✧ The assay requires less amount of harmful solvent than instrument analyses.
- ✧ With ease of handling, the total time for measurement is only 2.5 hours.
- ✧ The kit, a 96-well microplate format, enables simultaneous measurement of multiple samples at more reasonable cost.

Measuring Principle

1. Competitive Reaction

The test is based on the recognition of LAS by specific monoclonal antibodies. LAS present in the sample and a LAS-enzyme conjugate (i.e. LAS labeled with a coloring enzyme :HRP) are premixed and added into each well of a microplate, and allowed to compete for limited number of binding sites of specific antibodies immobilized on the surface of the wells. When the LAS concentration is higher relative to the enzyme conjugate, the LAS will predominantly bind the antibody and vice versa.

2. Chromogenic Reaction

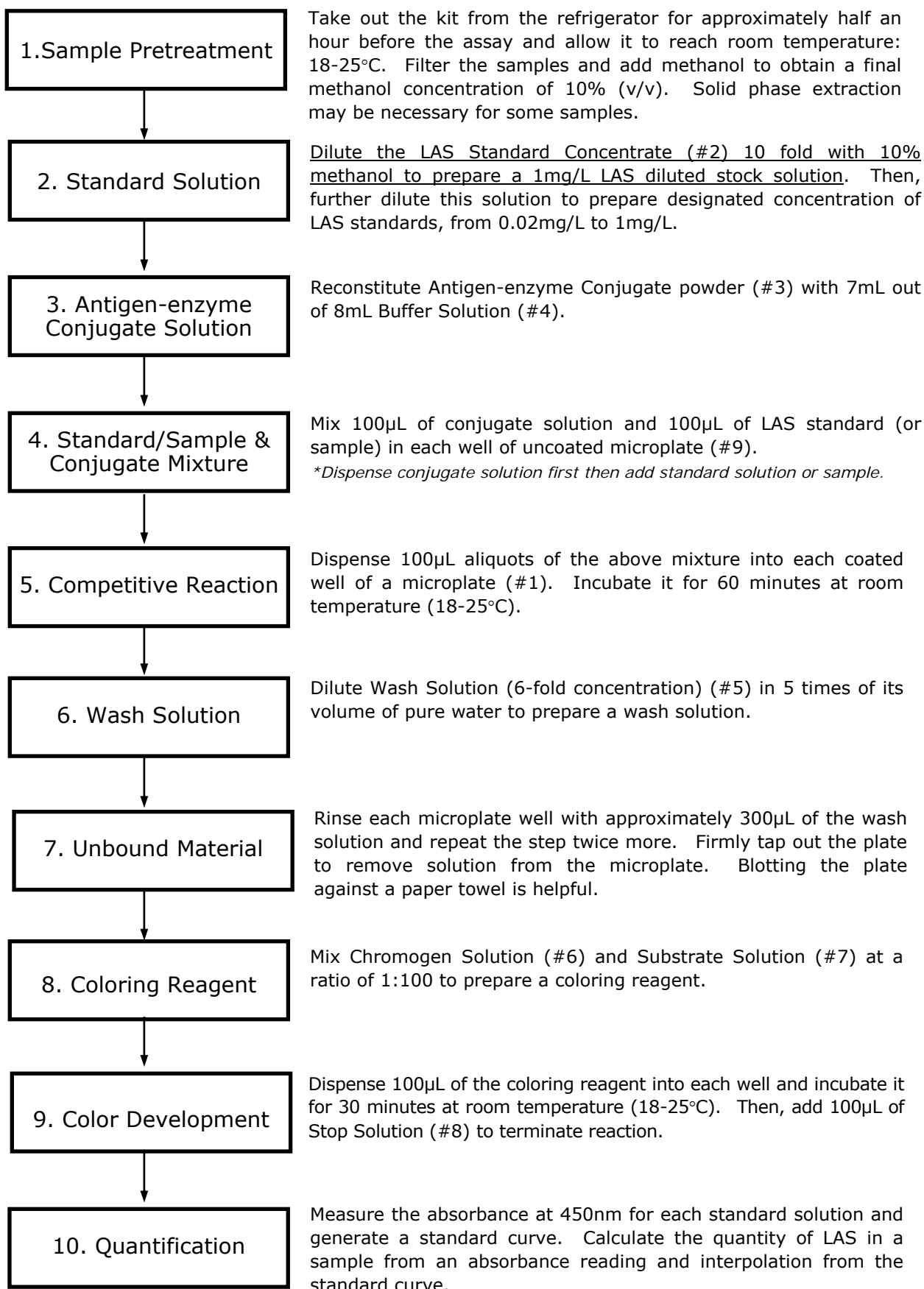
Unbound LAS and excess LAS-enzyme conjugates are washed out. The presence of LAS is detected by adding a chromogenic substrate :TMB. The enzyme-labeled LAS bound to the LAS antibody in the plate, catalyzes the conversion of the substrate to a colored product. After an incubation period, the reaction is stopped by the addition of a diluted acid. The higher the LAS concentration in a sample, for example, leads to less antigen-enzyme conjugate bound to the antibody binding sites in a microplate well, generating a lighter color, i.e. lower absorbance.

3. Quantitative Analysis

The standard curve, a dose-response curve obtained from known concentrations of LAS standards, is determined from the absorbance at 450nm. The LAS concentration in each sample is accurately calculated by interpolation using the absorbance intensity obtained from the standard curve.

Flowchart for LAS Measurement

<Please follow the steps described in Test Protocol (PP6-9)>



Kit Content

#	Contents	Volume	Quantity	Storage
1	MoAb-Coated Microplate	96 Wells	1 Plate	2-8°C
2	LAS Standard Concentrate (C12, 10mg/L, 10% Methanol)	4mL	1 Vial	2-8°C
3	Antigen-enzyme Conjugate powder		2 Vials	2-8°C
4	Buffer Solution	8mL	2 Vials	2-8°C
5	Wash Solution (6-fold concentration)	50mL	1 Vial	2-8°C
6	Chromogen Solution	250µL	1 Vial	2-8°C
7	Substrate Solution - <i>red marker</i> -	15mL	1 Vial	2-8°C
8	Stop Solution	15mL	1 Vial	2-8°C
9	Uncoated Microplate	96 Wells	1 Plate	---
10	Adhesive Plate Cover	---	1	---
11	Instruction Booklet	---	1	---

Other Essential Reagents/Materials

Essential - When Sample Concentration is NOT Required.

1. Glass disposable test tubes (e.g. IWAKI, item No. 9831-1207)
*Be sure to use disposable tubes to avoid LAS adsorption.
2. Glass fiber filters (e.g. ADVANTEC Co., item No. 36481047 Φ 47mm) and filtering equipment
3. Micropipettes (20µL - 200µL and 100µL - 1000µL, e.g. Gilson Pipetman P-200, P-1000) and tips
4. Multichannel pipettes :50µL - 300µL (e.g. Finnpipette Digital 8-channel Pipettor) and tips
5. Microplate reader (450nm wavelength) (e.g. TECAN Sunrise Remote)
6. Stop watch
7. Strip ejector (e.g. COSTAR, No.2578)
8. Methanol (HPLC grade)
9. Pure water

Essential - When Sample Concentration through SPE is Required.

- 1-9. the same as above
10. Solid phase extraction cartridge (e.g. J.T. Baker SPE Column C18, cat # 562-20014; Bond Elut C18 Octadecyl, cat # 5010-11024; Sep-Pak PS-2 N20131, Waters)

IMPORTANT

- Comparative tests should be performed if an alternate supplier is used for specified reagents or materials.

Test Protocol

IMPORTANT

- For research use only, not for human use.
- Take out all the kit contents from the refrigerator and let them reach room temperature (18-25°C) for approximately 30 minutes prior to the assay.
- Do not mix reagents from different kits.
- Store reagents under refrigeration (2-8°C)
- Do not use expired kits.
- Dispose of kit components in accordance with applicable regulations after use.
- Duplicate measurement is recommended for more accurate determination.

CAUTION

Wear appropriate protective clothing, gloves and eyewear to avoid any accidental contacts.

1. Sample Pretreatment

Clear Sample: Add 100% methanol to be 10% (v/v) methanol solution. Confirm the pH of the filtrate is between 5 and 8. If pH is out of this range, add acid or base to adjust pH.

Turbid Sample: Filter the sample through a glass fiber filter (To save time, suction with a vacuum pump is recommended). Wash the residue, if any, with 100% methanol (Make sure the amount of methanol does not exceed 1% of the total volume of the filtrate). Adjust methanol content of the filtrate to be 10% (v/v). Confirm the pH of the solution as described in "Clear Sample."

Sample with Low LAS: Concentrate the sample with solid phase extraction as follows.

- 1) Pour 5 ml methanol followed by 5 ml pure water through a solid phase extraction column.
- 2) Confirm the pH of the filtrate is between 5 and 8. If pH is out of this range, add acid or base to adjust pH.
- 3) Pressurize or vacuum the column to adjust a flow rate at 10-20ml/minute.
- 4) Wash the column with 10 ml pure water and then dry it with vacuuming or nitrogen gas.
- 5) Elute the analyte with 10 ml of 100% methanol.
- 6) Dilute the eluant with pure water to prepare 10% methanol solution. If concentration is necessary, evaporate the eluant and dissolve the residue in 10% methanol.

This sample pretreatment method is also applicable to Alkylphenol Ethoxylate (APE) and AE (Alcohol Ethoxylate) ELISA kits.

2. Standard Solution

- 1) 10-fold Diluted Stock

!! IMPORTANT !!

First, dilute the 10mg/L LAS concentrate solution (#2, 10% v/v methanol) with 9 times of its volume of 10% v/v methanol to prepare the 1mg/L LAS diluted stock solution.

- 2) LAS Standard Preparation

Dilute the 1mg/L solution, prepared above, in 10% methanol to obtain LAS from 0.02mg/L to 1mg/L, which represents the dynamic range of this kit. The following is an example.

Standard solution	(mg/L)	1	0.1	0.02	0
1mg/L LAS concentrate	(μL)	1000	100	20	0
10% methanol	(μL)	0	900	980	1000
Total	(μL)	1000	1000	1000	1000

※If you miss 10-fold dilution step to prepare standard solutions directly from the

10mg/L LAS concentrate (#2), the resulting concentration is 10 times as high as that of originally anticipated and the absorbance reading is lowered around 0.2-0.5. Be sure to dilute the concentrate 10 fold as the first step.

- Prepare the standard LAS solution just before the test. Standard solutions, once diluted from the concentrate, are NOT reusable at a later date. Prepare new standard solution for every test session.
- Disposable glass tubes are recommended for dilution to minimize adsorption and contamination.
- In order to minimize LAS adsorption on the walls of the tube, be sure to dispense 10% methanol first in a tube and then add 10mg/L LAS concentrate (#2) to prepare 1mg /L solution.
- Dilute directly from 1mg /L LAS stock solution to prepare the designated concentration of LAS (0.02, 0.1mg/L) to minimize LAS adsorption on the wall.
- Mix by filling the tip and expelling the contents with a pipette. Do not stir vigorously, with a Vortex mixer for example to prevent its foaming and non-specific adsorption onto the test tube surface.
- Be sure the standard concentrate is tightly capped after use and store it in a refrigerator. The standard solution must also be sealed or capped tightly to avoid methanol evaporation.
- Keep the methanol concentration of standard solutions at 10%. Higher methanol content in the sample may damage antibody and lower content may result in inaccurate readings.
- Do not discharge any untreated waste liquid. For example, soak cloth or paper in fluid for incineration. Dispose according to local, state or federal regulations.

3. Antigen-enzyme Conjugate Solution

Reconstitute a bottle of antigen-enzyme conjugate powder (#3) with 7mL out of 8 mL buffer solution (#4) to prepare antigen-enzyme conjugate solution.

- Store the conjugate solution at 2-8°C; it will be stable for approximately 2 weeks. 7mL is sufficient for approximately 50 wells.
- Mix by filling the tip and expelling the contents with a pipette. Be sure not to generate bubbles when you transfer liquid.
- Mix a pair of reconstituted solutions when you use them altogether.

4. Mixture of Standard/Sample and Conjugate Solution

Transfer 100µL of conjugate solution, and then transfer 100µL of LAS standard, prepared in Section 2-2), or 100µL of sample, prepared as 10 % (v/v) methanol solution into each well of the uncoated microplate (#9) and mix by filling the tip and expelling the contents with a pipette.

- Dispense conjugate solution first, then add standard solution or sample to avoid non-specific adsorption on the inner surface of the well.
- Mix by filling the tip and expelling the contents with a pipette. Be sure not to generate bubbles when you transfer liquid.
- Use 10% methanol as a blank.

5. Competitive Reaction

Dispense 100µL aliquots of the mixture, prepared in the above Section 4, into each coated well of the microplate (#1). Tap the plate lightly to make the liquid level horizontal. Incubate the microplate for 60 minutes at room temperature (18-25°C).

- Split the microplate, with a strip ejector for example, to use the necessary number of wells. This microplate is breakable into 12 strips, each of which consists of 8 wells. Place back the unused plate strips in the packet, seal and store them at 2-8°C.
- Be sure not to generate bubbles when you transfer liquid to avoid erroneous reading. To remove them, tap the plate lightly.
- Cover a microplate with film to avoid contamination and evaporation.
- Do not move or shake a microplate during the reaction.
- A temperature-controlled bath (18-30°C) is recommended.
- Secure the constant reaction time for each well, particularly to measure multiple

samples.

6. Wash Solution

Dilute Wash Solution (6-fold concentration) (#5) in 5 times of its volume of pure water to prepare a wash solution, e.g. 20mL of concentrate and 100mL of pure water.

- Prepare the necessary amount of solution if you plan to run assays on different days with a split plate. The rule of thumb is 1.2mL of wash solution is required per well, i.e. approximately 120 mL for a whole plate.
- The wash solution must be stored at 2-8°C; it will be stable approximately for a month after preparation.

7. Unbound Material

Rinse each microplate well with approximately 300µL of the wash solution and repeat the step twice more. Then, firmly tap out the plate to remove solution from the microplate. Blotting the plate against a paper towel, a clean cloth or a lint-free towel is helpful.

- Be sure to remove any remaining solution, which may cause a measurement error.
- Be sure the bottom of the plate is free from any fingerprints or dirt. Otherwise absorbance readings will be significantly altered.
- Do not discharge any untreated waste liquid. For example, soak cloth or paper in fluid for incineration.

8. Coloring Reagent

Mix Chromogen Solution (#6) and Substrate Solution (#7, a red mark on a white cap) at a ratio of 1:100 to prepare the coloring reagent, e.g. add 120µL of Chromogen Solution (#6) to 12 mL of Substrate Solution (#7) while stirring gently with a pipette tip.

- Prepare the coloring reagent within 15 minutes before the reaction.
- Dispense Substrate Solution first and then add Chromogen Solution.
- Prepare the mixture to the minimum necessary. 1mL of mixture is enough for 8 wells; approximately 12 mL is necessary for the whole plate. Screw the caps tightly and keep them in a refrigerator.
- The solution cannot be stored even under refrigeration.

9. Color Development

Dispense 100µL of the coloring reagent mixture, prepared in Section 8, into each microplate well and incubate the microplate for 30 minutes at room temperature (18-25°C). Then, add 100µL of Stop Solution (#8) to terminate the reaction.

- A temperature-controlled bath (18-30°C) is recommended.
- Secure the constant reaction time for each well, particularly to measure multiple samples.
- Each well colored with a blue color from the coloring reagent will turn yellow once the stop solution is added.

10. Quantification

Read the absorbance at 450nm for each standard solution and samples with a plate reader.

- Measure the absorbance within 15 minutes after the reaction is stopped.
- Prepare a standard curve based on at least duplicate standards for every assay.
- Be sure the bottom of the plate is free from any fingerprints or dirt. Otherwise absorbance readings will be significantly altered.
- The assay must be performed within the range between 0.02mg/L and 1mg/L. Samples of concentration beyond 1mg/L must be diluted with 10% methanol and re-tested. If the concentration of LAS in a sample is completely unknown, more than one dilution of each pretreated sample is recommended to be included in the assay. Several options are available for the calculation of the LAS concentration in samples.

(1) Computer aided Calculation

Calculate using microplate analysis software.

A 4-parameter logistic fitting software is recommended, for example " Delta Soft " from BioMetallics, Inc., Princeton, NJ (<http://www.microplate.com>).

(2) Graph Paper (Section Paper) aided Fitting

Calculate using Log-Log (or Log-Linear) Graph Paper (Section Paper) Fitting.

X-axis : LAS concentration

Y-axis : Optical Density(OD) or Inhibition Rate(B/B0%)

$$\text{Inhibition Rate(B/B0\%)} = (\text{Sample or standard OD})/(\text{OD at LAS standard}=0)$$

(Example)

Standard OD or B/B0%

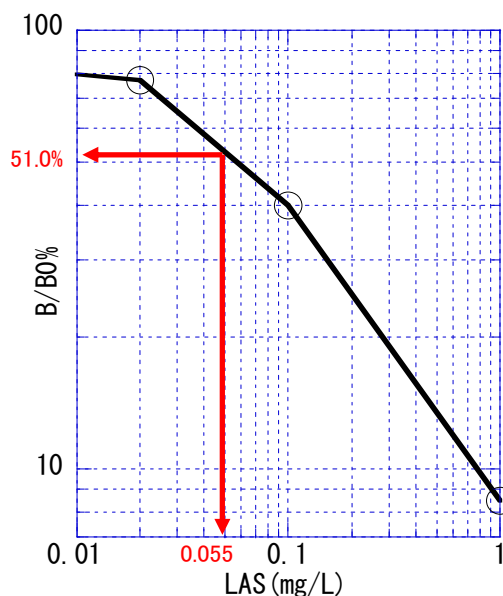
LAS(mg/L)	OD	B/B0%
0	1.312	100
0.02	1.010	77
0.1	0.525	40
1.0	0.105	8

Example:

LAS(mg/L)	OD	B/B0%
0.055	0.669	51.0

Log-Log Graph Paper Calculation

LAS=0.055(mg/L) from B/B0%=51.0%



APPENDIX

1. Plate Layout

LAS MoAb-Coated Microplate has 96 wells breakable into 8 x 12 strips.

Example 1) Full Plate Format

Four different concentrations of LAS standards (0, 0.02, 0.1, 1mg/L) are assayed in duplicates. The standards take up 8 wells, leaving the rest of 88 wells for samples. With duplicate measurement, the whole plate can take 44 samples altogether.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	S5	S5	S13	S13	S21	S21	S29	S29	S37	S37
B	0.02	0.02	S6	S6	S14	S14	S22	S22	S30	S30	S38	S38
C	0.1	0.1	S7	S7	S15	S15	S23	S23	S31	S31	S39	S39
D	1.0	1.0	S8	S8	S16	S16	S24	S24	S32	S32	S40	S40
E	S1	S1	S9	S9	S17	S17	S25	S25	S33	S33	S41	S41
F	S2	S2	S10	S10	S18	S18	S26	S26	S34	S34	S42	S42
G	S3	S3	S11	S11	S19	S19	S27	S27	S35	S35	S43	S43
H	S4	S4	S12	S12	S20	S20	S28	S28	S36	S36	S44	S44

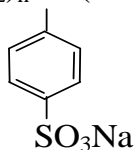
Example 2) Partial Plate Format

Four different concentrations of LAS standards are assayed in duplicates. The plate is split into two for independent assays. Half a plate can take up to 20 samples with duplicate measurement.

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	S5	S5	S13	S13	0	0	S5	S5	S13	S13
B	0.02	0.02	S6	S6	S14	S14	0.02	0.02	S6	S6	S14	S14
C	0.1	0.1	S7	S7	S15	S15	0.1	0.1	S7	S7	S15	S15
D	1.0	1.0	S8	S8	S16	S16	1.0	1.0	S8	S8	S16	S16
E	S1	S1	S9	S9	S17	S17	S1	S1	S9	S9	S17	S17
F	S2	S2	S10	S10	S18	S18	S2	S2	S10	S10	S18	S18
G	S3	S3	S11	S11	S19	S19	S3	S3	S11	S11	S19	S19
H	S4	S4	S12	S12	S20	S20	S4	S4	S12	S12	S20	S20

2. Chemical Structure of LAS Standard

Linear Alkylbenzenesulfonate (LAS)



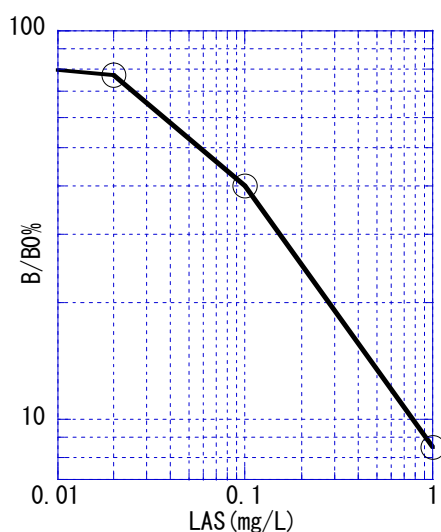
$$n + m = 9$$

3. Cross-reactivity Pattern

Compound	% reactivity
Anionic surfactants	
Linear alkylbenzene sulfonate (LAS)	
C12	100
C11	87
C10	101
C9	114
C8	75
Sodium dodecyl sulfate (SDS)	1.0
Sodium myristate	0.3
Sodium laurate	0.1
Sodium palmitate	<0.1
Sodium stearate	<0.1
Nonionic surfactants	
Nonylphenol ethoxylate (EO=10)	<0.1
Polyoxyethylene (EO=20) sorbitan monolaurate	<0.1
Disodium lauryl sulfosuccinate	<0.1
Benzenesulfonic acid	<0.1
Phenol	<0.1
Toluene	<0.1
Xylene	<0.1

The monoclonal antibody has a high specificity to LAS with various alkyl chain length (n=8-12) and doesn't cross-react with other surfactants or compounds of similar structure.

4. LAS Standard Curve



This test kit has a wide detection range between 0.02mg/L and 1mg/L. Samples within this range can be directly applied to assay only after filtration.

Samples outside the upper limit must be diluted with 10% methanol. Samples with LAS content below the range must be concentrated with solid phase extraction prior to the ensuing session.

Coefficient of variation (CV) is generally under 10% throughout the dynamic range.



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