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BRAXIS

Ecologiena®

LAS ELISA KIT

(Microplate)

User's Guide

Kit Feature

- ✧ Linear Alkylbenzene Sulfonate (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) 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. 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.

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%MeOH)	4mL	1 Vial	2-8°C
3	Antigen-enzyme Conjugate	7mL	2 Vials	2-8°C
4	Buffer Solution ***– white cap –***	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 ***– red marker –***	15mL	1 Vial	2-8°C
8	Stop Solution ***– black cap –***	15mL	1 Vial	2-8°C
9	Uncoated Microplate	96 Wells	1 Plate	---
10	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 Φ47mm) and filtering equipment
3. Micropipettes (20µL - 200µL and 100µL - 1000µL, e.g. Gilson Pipetman P-200, P-1000) and tips (e.g. ICN Superpack 96NS)
4. Multichannel pipettes :50µL - 300µL (e.g. LabSystems Finnpipette Digital 8-channel Pipettor) and tips (e.g. ICN Superpack 96NS)
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)

Essential - When Sample Concentration through SPE is Required.

1-8. the same as above

9. Solid phase extraction cartridge (e.g. J.T. Baker SPE Column C18, cat # 562-20014; Bond Elut C18 Octadecyl, cat # 5010-11024)

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.

- 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 10mg/L LAS concentrate (#2) first in a tube and then add 10% methanol to prepare 1mg /L solution.
- Dilute directly from 1mg /L LAS stock solution to prepare the designated concentration of LAS (0.02, 0.05, 0.1, 0.4mg/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, white cap) 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 LAS standard, prepared in Section 2-2), or 100µL of sample, prepared as 10 % (v/v) methanol solution, and then transfer 100µL of conjugate solution into each well of the uncoated microplate (#9) and mix by filling the tip and expelling the contents with a pipette.

- Dispense standard solution or sample first, then add conjugate solution 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.

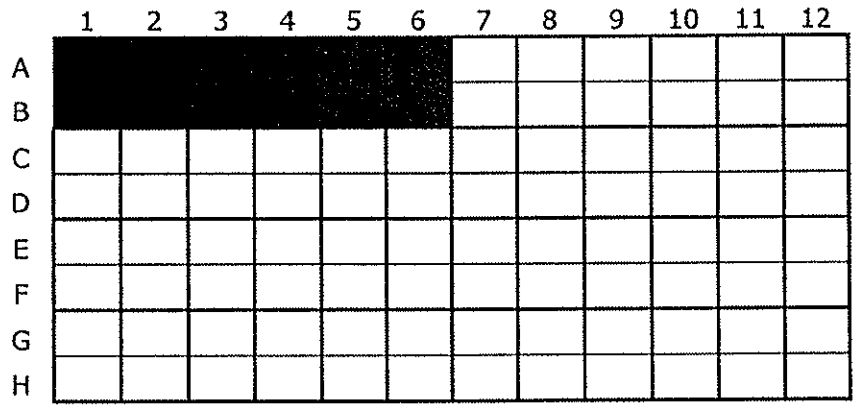
APPENDIX

1. Plate Layout

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

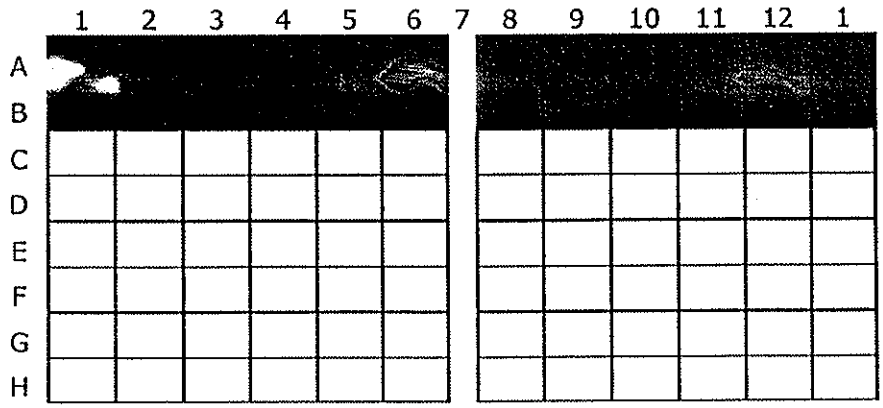
Example 1) Full Plate Format

Six different concentrations of LAS standards (0, 0.02, 0.05, 0.1, 0.4, 1mg/L) are assayed in duplicates. The standards take up 12 wells, leaving the rest of 84 wells for samples. With duplicate measurement, the whole plate can take 42 samples altogether.



Example 2) Partial Plate Format

Six 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 18 samples with duplicate measurement.



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