Ecologiena

Nonionic Surfactant

APE ELISA KIT

(Microplate)

User's Guide

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LIMITED WARRANTY

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

Measuring Principle (Competitive ELISA)

1. Competitive Reaction

The test is based on the recognition of APE by specific monoclonal antibodies. APE present in the sample and an APE-enzyme conjugate (i.e. APE 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 APE concentration is higher relative to the enzyme conjugate, the APE will predominantly bind the antibody and vice versa.

2. Chromogenic Reaction

Unbound APE and excess antigen-enzyme conjugates are washed out. The chromogenic substrate is then added to develop color in conjunction with the enzyme conjugate. The amount of APE-enzyme conjugate remaining bound to the antibody will determine the color intensity. The higher APE concentration in 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 APE standards, is determined from the absorbance at 450nm. The APE 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	APE Standard Concentrate (NP10EO, 4mg/L 20%MeOH)	4mL	1 Vial	2-8°C
3	Antigen-enzyme Conjugate	for 7mL	2 Vials	2-8°C
4	Buffer Solution– *white cap*	8mL	2 Vials	2-8°C
5	Uncoated Microplate	96 Wells	1 Plate	---
6	Wash Solution (6-fold concentration)	50mL	1 Vial	2-8°C
7	Chromogen Solution	250µL	1 Vial	2-8°C
8	Substrate Solution– *red marker*	15mL	1 Vial	2-8°C
9	Stop Solution– *black cap*	15mL	1 Vial	2-8°C
10	Instruction Booklet	---	1	---

Other Essential or Recommended Reagents/Materials

Essential

1. Disposable test tubes (e.g. IWAKI, item No. 9831-1207)
2. Glass fiber filters (e.g. ADVANTEC Co., item No. 36481047 ϕ47mm) and filtering equipment
3. Micropipettes (20µL -100µL and 100µL -1000µL) and tips
4. Multichannel pipettes (50µL -300µL) and tips
5. Microplate reader (450nm wavelength) (e.g. TECAN, SPECTRA Classic)
6. Stop watch

7. Methanol (for HPLC grade)

Recommended

8. Plate cover
9. Strip ejector (e.g. COSTAR, No.2578)

If Needed(Solid Phase Extraction)

10. Solid Phase Extraction Cartridge(e.g. J.T. Baker SPE Column C18 #7020-06, VARIAN Bond Elut™ C18 Octadecyl, 3M Empore™ Disk SDB-RPS etc.)
11. 1M Acetic buffer acid (pH5)

IMPORTANT

- Comparative tests should be needed 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 a refrigerator and let them reach room temperature (18-25°C) prior to the assay.
- Do not mix reagents from different kits.
- Store reagents under a condition as defined in this guide.
- Do not use expired kits.
- Dispose of kit components in accordance with applicable regulations after use.

CAUTION

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

1. Sample Filtration

Filter raw water samples through the specified glass fiber filter (1µm pore diameter).

2. Solid Phase Extraction

If an extraction and concentration process is not necessary, add methanol to the filtrate to be at a final methanol concentration of 10% (v/v). If not, prepare each sample by the extraction procedure.

[Example]

1) Acidify filtered sample with 1M Acetic buffer acid (pH5).
2) Rinse a C18 solid phase cartridge with methanol and then with distilled water.
3) Pour sample liquid through the cartridge.
4) Wash the cartridge with distilled water and elute APE with methanol.
5) Evaporate the solvent with nitrogen.
6) Dissolve the residue in 10% methanol.

3. Standard Solution

Dilute 4mg/L APE concentrate solution (20% methanol) with methanol and/or distilled water and prepare 10% methanol solution containing APE from 0.02mg/L to 1mg/L. Following is an example.

Standard solution	(mg/L)	1.0	0.5	0.2	0.1	0.05	0.02	0
4mg/L APE concentrate	(µL)	250	125	50	25	25	20	0
Methanol	(µL)	50	75	90	95	195	396	100
Distilled water	(µL)	700	800	860	880	1780	3584	900
Total	(mL)	1.0	1.0	1.0	1.0	1.0	1.0	1.0

- Pour concentrate first into a disposable tube, then add methanol and finally distilled water for dilution to avoid non-specific adsorption onto the tube surface.
- Prepare the standard APE solution just before the test. Standard solution, once diluted from the concentrate, is NOT reusable at a later date. Prepare new standard solution for every test session.
- 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.
- Dilute the concentrate in a single step to minimize adsorption onto the tube surface.
- Mix by filling the tip and expelling the contents with a pipette. Do not stir vigorously, with a Vortex mixer for example. Otherwise foaming may generate incorrect optical density.

4. Antigen-enzyme Conjugate Solution

Reconstitute antigen-enzyme conjugate powder with 7mL of buffer solution to prepare antigen-enzyme conjugate solution.

- Store the conjugate solution at 2-8°C; it will be stable for approximately 2 weeks.
- Mix by filling the tip and expelling the contents with a pipette.
- Mix a pair of reconstituted solutions when you use them altogether.

5. Mixture of Standard/Sample and Conjugate Solution

Transfer 100µL of APE standard (or sample) and 100µL of conjugate solution into each well of the uncoated microplate and mix by filling the tip and expelling the contents with a pipette.

- Dispense standard solution first, and then add conjugate solution to avoid non-specific adsorption on the inner surface of the well.
- Use 10% methanol solution as a blank.

6. Competitive Reaction

Dispense 100µL aliquots of the above mixture into each coated well of the microplate. Incubate the microplate for 60 minutes at room temperature (18-25°C).

Split the microplate, with a strip ejector for example, to use 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.

- Place the pipette tip on the well bottom and slowly transfer the content so that any mixture solution does not touch or remain on the non-coated area of the well.
- Cover a microplate with film to avoid contamination.
- Do not move or shake a microplate during the reaction.
- Be sure not to generate bubbles when you transfer liquid to avoid erroneous reading. To remove them, tap a plate lightly.
- Secure the constant reaction time for each well, particularly to measure many samples.

7. Wash Solution

Dilute 6-fold wash solution concentrate with distilled water at a ratio of 1:5 to prepare a wash solution. Rinse each well with approximately 300 μL of the solution and repeat 3 times. Be sure that all the wash solution is removed from the microplate prior to the assay.

- The wash solution must be stored at 2-8°C; it will be stable approximately for a month after preparation.

8. Chromogenic Reaction

Mix chromogen solution and substrate solution (with a red mark on a white cap) at a ratio of 1:100 to prepare the mixture solution. Dispense 100μL of the mixture into each microplate well and incubate the microplate for 30 minutes at room temperature (18-25°C). Then, add 100μL of stop solution (with a black cap) to stop the reaction.

- Prepare the mixture solution within 15 minutes before the reaction. The solution cannot be stored even under refrigeration.
- Secure the constant reaction time for each well, particularly to measure many samples.

9. Quantification

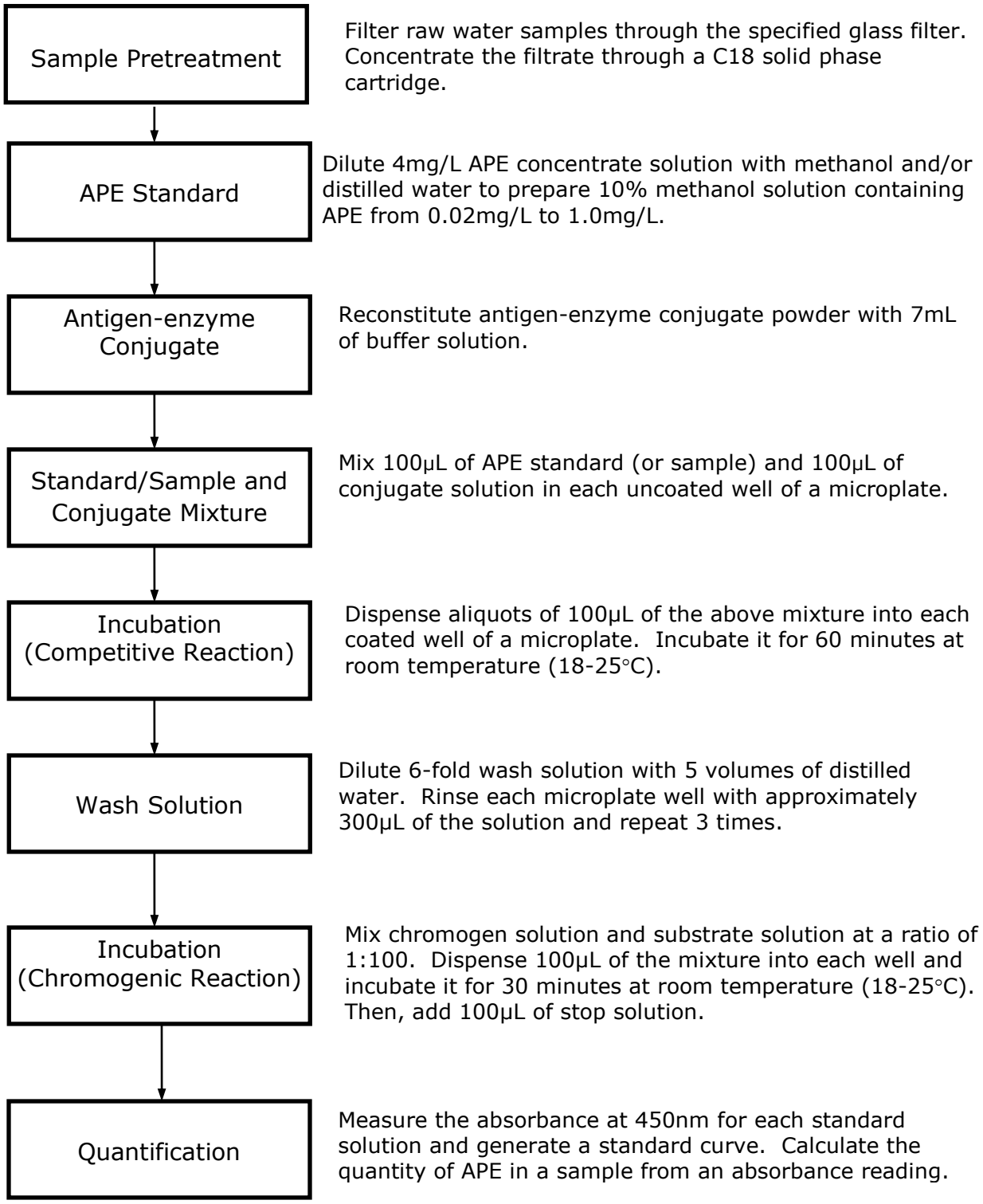
Measure the absorbance at 450nm for each standard solution and generate a standard curve. Calculate the quantity of APE in a sample from an absorbance reading.

- Measure the absorbance within 15 minutes after the reaction is stopped.
- Prepare a standard curve based on at least duplicate standards for every assay.
- The assay must be performed within the range between 0.02mg/L and 1.0mg/L. Samples of concentration beyond 1mg/L must be diluted with 10% methanol and re-tested.

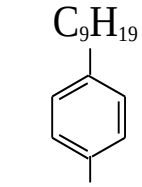
APPENDIX

1. Flowchart for APE Measurement

Note: Take out all the kit contents from a refrigerator and let them reach room temperature (18-25°C) prior to the assay.
<Please follow the steps describing in the text: Test Protocol>



2. Chemical Structure of APE Standard



$(OCH_2CH_2)_nOH$ n=10 (Ave. EO chain length)

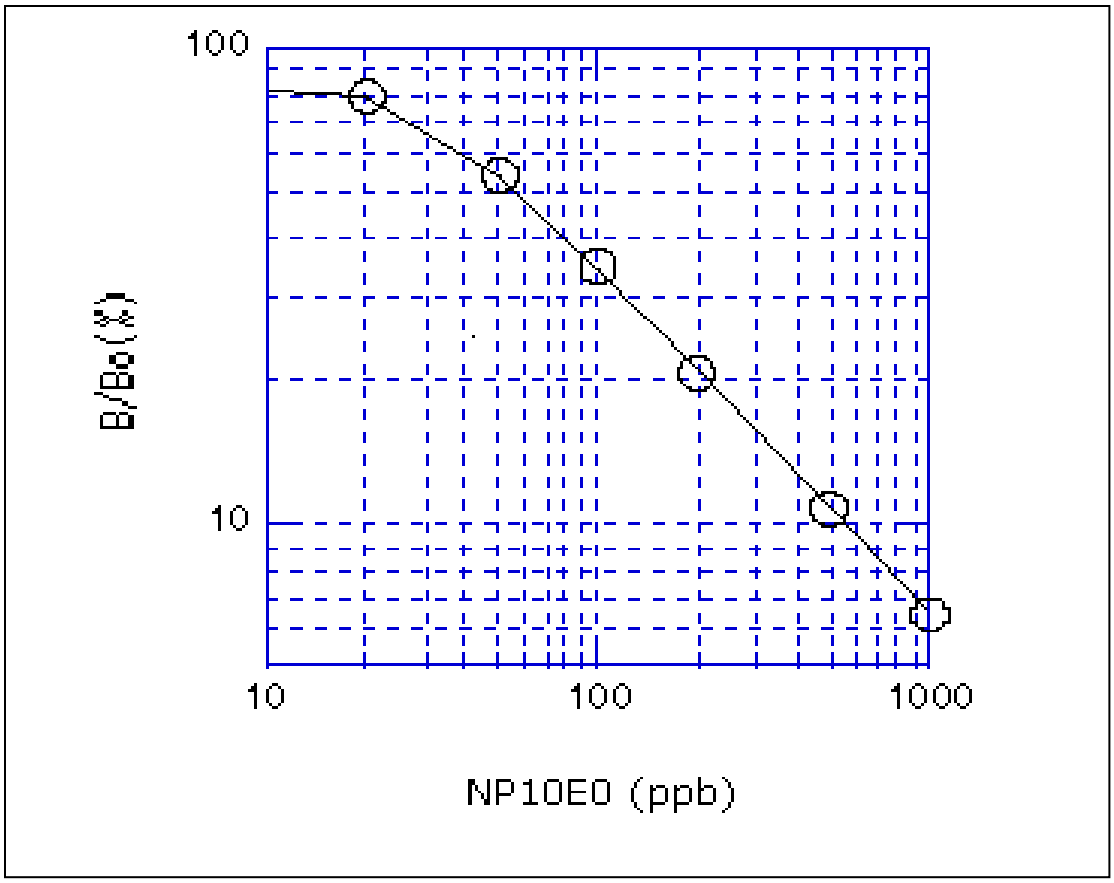
3. Cross-reactivity Pattern

The monoclonal antibody has a high specificity to APE with various polyethoxylic chain length (n=1-22) and doesn't cross-react with other surfactants or compounds of similar structure.

Compound		%reactivity
Nonionic surfactants		
NPE (Nonylphenol ethoxylate)		
NP20EO	(Ave. EO chain length 20)	79
NP15EO	(Ave. EO chain length 15)	73
NP10EO	(Ave. EO chain length 10)	100
NP5EO	(Ave. EO chain length 5)	82
NP2EO	(Nonylphenol di ethoxylate)	32
NP1EO	(Nonylphenol mono ethoxylate)	14
OPE (Octylphenol ethoxylate)		
OP20EO	(Ave. EO chain length 20)	92
OP15EO	(Ave. EO chain length 15)	129
OP10EO	(Ave. EO chain length 10)	200
OP5EO	(Ave. EO chain length 5)	244
OP2EO	(Nonylphenol di ethoxylate)	129
NPnEC (Nonylphenol Carboxylic Acid)		
NPnEC (n=2)		267
NPnEC (n=1)		200
AE (Alkyl ethoxylate)		<0.2
Anionic surfactants		
Linear Alkylbenzene Sulfonates (LAS)		<0.2
Sodium Laurate (SOAP)		<0.2
Sodium Dodecyl Sulfate (SDS)		<0.2
Alkylether Sulfate (AES)		<0.2
Phenol		<0.2
Polyethylene glycol (PEG)		<0.2

4. APE Standard Curve

This test kit has a wide detection range between 0.02 mg/L and 1.0mg/L. Samples within this range can be directly applied to the assay only after filtration. Samples outside of the range must be either diluted with 10% methanol or extracted with solid phase concentration prior to analysis.



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