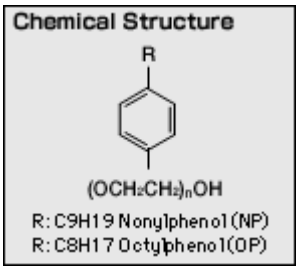
AP ELISA Kit

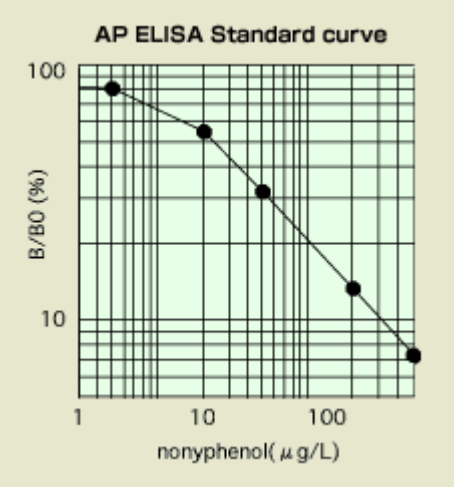
- The monoclonal antibody binds exclusively with Alkylphenol(AP), Alkylphenol Ethoxylate(APE), Nonylphenol Carboxylic acid(NPnEC) and does not cross-react with other surfactants or compounds of similar structure.
- The detection range is between 5µg/L and 500µg/L. A simple concentration protocol based on solid phase extraction is available to determine much lower concentration.
- 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.

AP

AP is suspected as one of the endocrine disrupting chemicals in many countries.
Octylphenol (n=8) and nonylphenol (n=9) are widely used as raw material of surfactants and anti-oxidants.
AP is generated by microbial decomposition of ethylene oxide chains in APE, which is widely used as nonionic surfactant.
NPnEC, another decomposed product of APE, is also assumed as hormone mimicking chemicals.



AP Standard Curve



Samples containing AP within the dynamic range (5µg/L- 500µg/L) can be directly applied to assay after filtration. Samples with AP 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.

Pretreatment (Simplified Solid Phase Extraction)

If an extraction and a concentration process is not necessary, add methanol to the filtrate to be a final DMSO concentration of 1% and methanol concentration of 10% (v/v). If not, prepare each sample by the extraction procedure.

[Example]

1. Rinse a PS-2 solid phase cartridge at 10ml/min. with methanol (5mL) and then with distilled water (10mL).
2. Pour sample liquid through the cartridge at 10mL/min.
3. Wash the cartridge with distilled water (10mL).
4. Dry the cartridge with nitrogen or centrifuge (3.000rpmx10min.).
5. Elute the analyte with methanol (10mL) then evaporate the solvent with nitrogen gas.
6. Dissolve the residue in hexane (0.5mL).
7. Pour the solvent through a silica gel cartridge (e.g. Sep Pak Vac Silica), conditioned with methanol/chloroform (20/80 v/v 10ml) and hexane (30ml).
8. Wash the cartridge with hexane (10mL).
9. Elute AP + APnEO (n=1-4) with chloroform (6mL) (Fraction A).
10. Elute APnEO (n=5 or more) with methanol/chloroform (20:80 v/v 5mL) (Fraction B)
11. Mix the Fraction A and B fractions and evaporate them with nitrogen gas. Dissolve the residue in DMSO and methanol solution to be a final concentration of 1% DMSO and 10% methanol.

Cross-reactivity Pattern

Compound	% reactivity
Nonylphenol	**100**
Octylphenol	**187**
Nonylphenol Ethoxylate (NPnEO)	
NP10EO (Ave. EO chain length 10)	100
NP7.5EO (Ave. EO chain length 7.5)	112
NP5EO (Ave. EO chain length 5)	140
NP2EO (Ave. EO chain length 2)	175
NP1EO (Nonylphenol mono ethoxylate)	127
OPE (Octylphenol ethoxylate)	
OP10EO (Ave. EO chain length 10)	156
Nonylphenoxy Carboxylic acid (NPnEC)	
NPnEC (n=2)	423
NPnEC (n=2)	423
Anionic Surfactants	
Linear Alkylbenzene Sulfonates (LAS)	1.6
Sodium Dodecyl Sulfate (SDS)	1.0
Alkylether Sulfate (AES)	<0.2
Sodium Laurate (SOAP)	0.3

Kit Format

Kit Format	Content	Comment
Microplate	96 wells & reagents	Needs a microplate reader(450nm) For multiple and simultaneous measurement