NPE ELISA Kit

Product #: E6008

Manufacturer Information

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1. Description

Nonylphenol polyoxyethylene ether (NPE) is used in detergents, paints, pesticides, personal care products, and plastics. Nonylphenol has attracted attention due to its prevalence in the environment and its potential role as an endocrine disruptor and xenoestrogen, due to its ability to act with estrogen-like activity.

This ELISA Kit is based on indirect competitive ELISA to detect NPE in raw milk within 25 min.

2. Principle

This ELISA Kit is based on indirect competitive ELISA, the coupled antigen was pre-coated in the microwells, the NPE will compete the antibody with the pre-coated antigen, then add enzyme conjugate and TMB substrate successively, the OD value of the sample is negatively correlated with the content of NPE contained in the sample. The standard curve is fitted by regression and multiplied by the corresponding dilution ratio to obtain the content of NPE in the sample.

3. Application

This kit is applicable for quantitative determination of NPE in raw milk.

4. Kit components

1) Microtiter plate, 96wells, 1 plate
2) NPE standards x6 vials, 1mL/vial, 0, 5, 20, 50, 80, 200 ng/mL
3) Enzyme conjugated antibody, 7mL, with brown cap
4) TMB Substrate, 12mL, with brown cap
5) 20x Wash buffer, 50mL, with transparent cap
6) Stop solution, 7mL, with white cap

5. Instrument and material required

1) ELISA reader, with 450/630nm
2) Centrifuge
3) Balance, 0.0001g
4) Centrifuge tube, 2mL, 4mL
5) Vortex mixer
6) Micropipette, 20-200μL, 100-1000μL
7) Multi-channel pipette, 250μL

6. Reagent required

Deionized water, concentrated HCl

7. Reagents preparation

Wash buffer:

Dilute 20x wash buffer with deionized water, in the volume ratio of 1:19, for example, 10mL 20x wash buffer + 190mL deionized water, mix thoroughly.

This diluted wash buffer can be stored at 4°C for 1 month.

0.1M HCl:

Take 100mL of deionized water, then add 0.83mL of concentrated HCl, mix thoroughly.

Please return to room temperature before using those reagents. Please note that you should shake up those liquid reagents before using.

8. Sample preparation

8.1 Precautions before prepare samples:

1) Use disposable tips during the test. Change new tip for different sample / reagent.
2) Make sure all lab wares are clean and ready to use.
3) Prepared sample shall be analyzed immediately after dilution.

8.2 Raw milk

Take 2mL raw milk into a new centrifuge tube (4mL), add 100μL 0.1M HCl, mix thoroughly, then centrifuge at 4 °C for 5 min at 12000g. then remove the fat layer, take 1mL of supernate into a 2mL centrifuge tube, take 50μL/well for analysis.

Note: please remove the fat layer after centrifugation to avoid the interference of fat.

9. Notice and precautions before assay

1) Make sure the ELISA kit and all reagents are returned to room temperature (20-25 °C /68-77 °F). For example, keep these reagents and kits at room temperature for at least 30min.
2) Return unused kit components to 2-8 °C.
3) Washing step is important for the reproducibility of the kit, please follow this instruction carefully.
4) Cover the ELISA plate during all incubation. Avoid direct sunlight.

10. Assay procedures

1) Take needed microwells and zip rest in the zip-bag and return to 2-8 °C.
2) Layout the plate and record sample and standard well positions. It is recommended to run all tests in duplicates.
3) **Add sample/standard**: add sample/standard into the wells, 50ul per each, then add enzyme conjugated antibody, 50μL per well, shake gently and then cover the plate and incubate at **37 °C /98.6 °F for 15min**.
4) **Wash**: take out the plate and pour the liquid out. Use the diluted wash buffer to wash the plate, 250μL/well. Wash for 4-5 times with interval of 10s. The pour the liquid out and tap the plate against absorbent paper. Eliminate the air bubble in the wells with micropipette tip if the bubble exists.
5) **Coloration**: add TMB substrate, 100μL per well, and then cover the plate and incubate at **37 °C /98.6 °F for 10min**.
6) **Stop the reaction**: add stop solution, 50μL per well, shake gently and read the plate with ELISA reader at 450nm. Read the plate within 5min after adding stop solution.

11. Result Calculation

This kit is based on competitive ELISA, thus the OD values is inversely proportional to the NPE content contained in sample. With ELISA reader, a standard curve can be plotted with the ODs obtained. You can use the NPE standards to finish the standard curve, horizontal axis is the logarithm of the concentration of NPE standards, vertical axis is the OD percentage of standards. Please note that the sample concentration you get from the standard curve is the diluted sample.

$$\text{OD Percentage } (\%) = \frac{B}{B0}$$

B – mean OD of standards or samples

B0 – mean OD of first standard (0 μg/mL)

12. Specifications of the kit

1) Sensitivity: 5 ng/mL
2) Specificity: 100% to NPE
3) Limit of Detection: 5 ng/mL
4) Recovery: 100%±30%
5) Precision: C.V<10%.

13. Cautions and tips for the test

1) Lower room temperature, e.g., lower than 20 ◦C may cause lower OD values. Please make sure all reagent and kit components are returned to room temperature.
2) Wash step is vital for the reproducibility of the kit. Please wash according to the kit instruction. Do not let the plate dry during wash. Continue the next operations immediately after wash step.
3) Shake each reagent gently before use.
4) Stop solution is acidic, please handle with care.
5) Do not use expired kits and reagents. Do not mix the reagent and kits from different LOT.
6) The kit is stored at 2-8◦C(36-46◦F), do not freeze.
7) TMB substrate is sensitive to sunlight. Avoid direct sunlight.
8) If Standard 1 (0μg/mL) OD is lower than 0.5, please do not use. The kit may be expired or deteriorated.
9) The incubation is 37 ◦C /98.6 ◦F, lower or higher temperature will cause changes of OD and sensitivity of the kits, which may affect the result of the assay.

14. Storage and expiration

The kit is valid for 12months when stored at 2-8 ◦C. LOT and Expiry information are printed on the package.