Dibutyl phthalate DBP ELISA Test Kit

Product #: E6010-96T

Manufacturer Information

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1. Description

Dibutyl phthalate (DBP) is an organic compound that is commonly used as a plasticizer because of its low toxicity and wide liquid range. With the chemical formula C6H4(CO2C4H9)2, it is a colorless oil, although commercial samples are often yellow.

This dibutyl phthalate (DBP) ELISA Kit is based on indirect competitive ELISA to detect dibutyl phthalate (DBP) in goat/cow/camel raw milk.

2. Principle

This ELISA Kit is based on indirect competitive ELISA, the coupled antigen was pre-coated in the microwells, the DBP 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 DBP contained in the sample. The standard curve is fitted by regression and multiplied by the corresponding dilution ratio to obtain the content of DBP in the sample.

3. Application

This kit is applicable for quantitative determination of DBP in goat/cow/camel raw milk and milk poeder.

4. Kit components

1) Microtiter plate, 96wells, 1 plate
2) DBP Spiking standard,1mL/vial, 10 μg/mL
3) DBP standards x6 vials, 1mL/vial, 0, 0.025, 0.075, 0.15, 0.5, 1.5 μg/mL
4) Antibody solution, 7mL, with green cap
5) Enzyme conjugate, 7mL, with brown cap
6) TMB Substrate, 12mL, with brown cap
7) Stop solution, 7mL, with white cap
8) 20x Wash buffer, 50mL, with transparent cap
9) 2x sample buffer, 50mL, with transparent 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

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.

Sample buffer:

Dilute 2x sample buffer with deionized water, in the volume ratio of 1:1, for example, 10mL 2x sample buffer + 10mL deionized water, mix thoroughly.

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

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 Goat/cow/camel raw milk

Take 1ml sample into 5ml centrifuge tube, add 3ml anhydrous ethanol, vortex for 1min to mix, centrifuge at 12000rpm for 5min.Take 400ul upper clear layer and add 250ul sample buffer, vortex for 30s. Take 50ul of the mixed liquid for assay.

8.3 Goat/cow/camel milk powder

Take 1.0000 ± 0.0050g sample into 15ml centrifuge tube, add 8ml deionized water, vortex for 1min to dissolve. Take 1ml dissolved sample into 5ml centrifuge tube, add 3ml anhydrous ethanol, vortex for 1min to mix, centrifuge at 12000rpm for 5min.Take 400ul upper clear layer and add 250ul sample buffer, vortex for 30s. Take 50ul of the mixed liquid for assay.

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, enzyme conjugate and antibody**: add sample/standard into the wells, 50μL per each, then add enzyme conjugate antibody, 50μL per well, after that, add antibody solution, 50μL per well, shake gently and then cover the plate and incubate at **25 °C for 30min**.
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 **25 °C for 15min**.
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 DBP content contained in sample. With ELISA reader, a standard curve can be plotted with the ODs obtained. You can use the DBP standards to finish the standard curve, horizontal axis is the logarithm of the concentration of DBP 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: 0.025 μg/mL
2) Specificity:

Dibutyl phthalate……..100%

diisobutyl phthalate……72%

Benzyl butyl phthalate……29%

Di-n-pentyl phthalate……..17%

Other DBPs……<1%

3) Limit of Detection: 0.1625 μg/mL
4) Dilute factor:6.5
5) Recovery: 70%-120%
6) 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 25 °C, 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.