Version 26-10/24

User's Manual

Benzo(a)Pyrene ELISA Kit

REF **DEIA6831-2**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

The Benzo[a]pyrene (BAP) ELISA Test Kit provides a competitive enzyme immunoassay for the quantitative analysis of Benzo[a]pyrene in water, milk, serum.

Principles of Testing

The assay is based on a competitive colorimetric ELISA assay. The Benzo[a]pyrene has been coated in the plate wells. During the analysis, sample is added along with the primary antibody specific for the Benzo[a]pyrene. If the target is present in the sample, it will compete for the antibody, thereby preventing the antibody from binding to the drug attached to the well. The secondary antibody, tagged with a peroxidase enzyme, targets the primary antibody that is complexed to the drug coated on the plate wells. The resulting color intensity, after addition of substrate, has an inverse relationship with the target concentration in the sample.

Reagents And Materials Provided

1. BAP-coated Microtiter Plate: 1 × 96-well plate (8 wells × 12 strips), 2-8°C
2. 200 ppb BAP Standards: 0.5 mL, 2-8°C
3. Empty Vials for Standards: 6 vials, 2-8°C
4. Standards Dilution Buffer: 25 mL, 2-8°C
5. BAP Antibody #1: 6 mL, 2-8°C
6. HRP-Conjugated Ab #2: 11 mL, 2-8°C
7. 10× PBS: 15 mL, 2-8°C
8. 20× Wash Solution **: 28 mL, 2-8°C
9. Stop Buffer: 12 mL, 2-8°C
10. TMB Substrate: 12 mL, 2-8°C

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Vortex mixer
4. 10, 20, 100 and 1000 µL pipettes
5. Multi-channel pipette: 50-300 µL (Optional)
6. Methanol and Cyclohexane/n-hexane
7. Distilled water

Storage

Store all reagents at 2-8°C, and not be frozen or thawed. The product is valid for 12 months when the kit is properly stored.

Specimen Collection And Preparation

Be sure samples are properly stored. In general, samples should be refrigerated at 2-4°C for no more than 1-2 days. Freeze samples to a minimum of -20°C if they need to be stored for a longer period. Frozen samples can be thawed at room temps (20 – 25C / 68– 77F) or in a refrigerator before use.

1. **1× PBS**

   Dilute 10× PBS 1 + 9; e.g. 20 mL Washing Buffer + 180 mL distilled water.

2. **35% Methanol/65% PBS**

   Mix 3.5 volume of Methanol with 6.5 volumes of 1× PBS.

3. **Water/Milk/Serum**

   3.1 Take 1.5 mL of water sample in a centrifuge tube and adjust the pH to 6.5-7.5 with 0.1 M HCl or 0.1 M NaOH.

   3.2 Centrifuge the water sample for 5 minutes at 4,000 ×g at room temperature (20-25°C/68-77°F).

   3.3 Take out 0.1 mL of the supernatant to a new glass tube, add 0.9 mL of Standards Dilution Buffer. Vortex 30 seconds.

   3.4 Use 50 µL of the solution per well in the ELISA test.

   Note: Dilution factor: 10.

Reagent Preparation

All reagents should be brought up to room temperature before use.

All reagents should be mixed by gently inverting or swirling prior to use. Prepare volumes that are needed for the number of wells being run. Do not return the reagents to the original stock tubes/bottles. Using disposable reservoirs when handling reagents can minimize the risk of contamination and is recommended.

1. **Preparation of 1X Wash Solution**

Mix 1 volume of 20X Wash Buffer concentrate with 19 volumes of distilled water.

2. **Preparation of Benzo[a]pyrene Standard**

Mix the following solutions by serial dilution starting from the highest concentration to the lowest in glass vials. Make standards fresh each time performing the assay:

Work Standards	Volume of BaP Source	Volume of Standards Dilution Buffer
2 ppb	20 µL (of 200 ppb stock)	1980 µL
1 ppb	1000 µL (of 2 ppb dilution)	1000 µL
0.4 ppb	600 µL (of 1 ppb dilution)	900 µL
0.1 ppb	300 µL (of 0.4 ppb dilution)	900 µL
0.04 ppb	400 µL (of 0.1 ppb dilution)	600 µL
0 ppb	0 µL	50 µL

Assay Procedure

Component	Volume per Reaction	24 Reactions
BAP Antibody #1	50 µL	1.2 mL
HRP-Conjugated Ab #2	100 µL	2.4 mL
1X Wash Solution	2.0 mL	48 mL
Stop Buffer	100 µL	2.4 mL
TMB Substrate	100 µL	2.4 mL

1. Add 50 µL of each BAP Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 µL of each sample in duplicate into different sample wells.
3. Add 50 µL of BAP Antibody #1 each well and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 minutes at room temperature (20 - 25°C / 68 - 77°F).
5. Wash the plate 3 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
6. Add 100 µL of HRP-Conjugated Ab #2 each well. Incubate the plate for 30 minutes at room temperature (20 - 25°C / 68 - 77°F) (Avoid direct sunlight and cold bench tops during the incubation. Covering the microtiter plate while incubating is recommended).
7. Wash the plate 3 times with 250 µL of 1X Wash Solution. After the last wash, invert the plate and gently tap the plate dry on paper towels (Perform the next step immediately after plate washings. Do not allow the plate to air dry between working steps).
8. Add 100 µL of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating (Do not put any substrate back to the original container to avoid any potential contamination. Any substrate solution exhibiting coloration is indicative of deterioration and should be discarded. Covering the microtiter plate while incubating is recommended).
9. fter incubating for 15 minutes at room temperature (20 - 25°C / 68 - 77°F), add 100 µL of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength (Before reading, use a lint-free wipe on the bottom of the plate to ensure no moisture or fingerprints interfere with the readings).

Calculation

A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

Relative absorbance (%) = absorbance standard (or sample) x 100 / absorbance zero standard

Use the mean relative absorbance values for each sample to determine the corresponding concentration of the tested drug in ng/mL from the Log/Log standard curve.

Sensitivity

Water/Milk/Serum: 0.5 ng/g or ppb

Specificity

Benzo[a]pyrene 100%

Hexachlorobenzene < 0.1%

Hexachlorocyclopentadine < 0.1%

Methoxychlor < 0.1%

Precautions

1. The standards contain BaP. Handle with particular care.
2. Do not use the kit past the expiration date.
3. Do not intermix reagents from different kits or lots except for components with the same part No's within expiration dates.
4. Add standards only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.

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