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Dexamethasone ELISA Kit

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(Catalog # E4942-100, 96 assays, Store at 4°C)

I. Introduction:

Dexamethasone is a synthetic glucocorticoid that is used to treat inflammatory conditions such as allergies, arthritis, lupus, and ulcerative colitis, numerous skin diseases, blood, eye, and kidney disorders. It is also given to patients undergoing chemotherapy to neutralize the side effects of antitumor treatments. Dexamethasone when combined with certain foods may cause drug-food interaction. Grapefruit and pomegranate juices increase the concentration of dexamethasone in the blood by interfering with the ability of hepatic enzymes to clear the drug from the body. Consumption of alcohol with dexamethasone may cause stomach ulcers. BioVision's Dexamethasone ELISA kit is used to quantitatively measure Dexamethasone in muscle tissue, milk, and feed samples. The kit is based on the Competitive ELISA principle. Samples and standards are added to the microwell plate that is pre-coated with an antigen and competes for binding to the anti-Dexamethasone antibody. The HRP conjugate is added to each well and any unattached conjugates are washed off using Wash Buffer. The HRP enzymatic reaction is detected by the addition of substrate reagents. Finally, the reaction is terminated with an acidic stop solution. The color developed is inversely proportional to the concentration of Dexamethasone in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Dexamethasone
Detection Limit: 0.2ppb for muscle tissue; 0.5ppb for milk; 1ppb for feed
Sensitivity: 0.1ppb
Cross reaction: Dexamethasone 100%

III. Sample Type:

Tissue, Feed, Milk

IV. Kit Contents:

Components	E4942-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4942-100-1
Standard (S0 – S5)	1 ml X 6	E4942-100-2
HRP Conjugate	11 ml	E4942-100-3
Antibody working solution	5.5 ml	E4942-100-4
Substrate A	6 ml	E4942-100-5
Substrate B	6 ml	E4942-100-6
Stop Solution	6 ml	E4942-100-7
Wash Buffer (20X)	40 ml	E4942-100-8
Reconstitution Solution (2X)	50 ml	E4942-100-9
Plate Sealer	3	E4942-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Ethyl acetate, NaOH, n-Hexane
- Microplate reader, nitrogen evaporator
- Clean eppendorf tubes and graduated cylinders for preparing standards or sample dilutions
- Absorbent paper

VI. Storage and Handling:

The entire kit may be stored at 4°C for up to 12 months from the date of shipment. Opened kit may be stable for 1 month at 4°C.

VII. Reagent and Sample Preparation:

Note: Bring all reagents to room temperature (20-25°C) 30 minutes before use.
Before using the kit, spin tubes and bring down all components to the bottom of tubes.

1. **Wash Buffer (1X):** Dilute 15 mL of **Wash buffer (20X)** with deionized water to 300 ml

2. **Standards Preparation:** Ready-to-use standards are as follows

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.1	0.3	0.9	2.7	8.1

3. **Sample Preparation:**
Note: The prepared sample maybe stored for up to one day at 2-8°C.

Sample pre-treatment: The following method must be used for pre-treatment of any kind of sample:
Note: Only the disposable tips can be used for the experiments and the tips must be changed when used for absorbing different reagents.

Solution preparation before sample pre-treatment:

1) **2M NaOH solution:** Dissolve 40 grams of NaOH to 500 ml with deionized water. Mix thoroughly.
2) **0.3M NaOH solution:** Add 75 ml of **2M NaOH solution** and make up the volume to 500 ml with deionized water. Mix thoroughly.
3) **Reconstitution Buffer (1X):** Dilute 1 part of **Reconstitution Buffer (2X)** with 1 part of deionized water. The 1X solution can be stored at 4 ^{0}C for 1 month.

Sample Preparation and pre-treatment (for Muscle tissue samples):

Detection limit: 0.2ppb

- Take 2 ± 0.05 grams of the homogenized muscle sample into a 50 ml centrifuge tube. Add 8 ml **Ethyl acetate**, mix for 5 mins, and centrifuge at 4000 r/min for 10 mins at room temperature (RT).
- Take 4 ml supernatant into a new 50 ml centrifuge tube, add 4 ml of **2M NaOH solution**, mix for 5 mins, centrifuge at 4000 r/min for 10 mins at RT
- Take 2 ml supernatant in a new 10 ml glass tube, dry by nitrogen evaporator or water bath at 50 – 60 ^{0}C
- After drying, dissolve the sample residue in 1 ml **Reconstitution Buffer (1X)**, mix for 2 mins. Take 100 µl for the analysis
- **Fold of dilution of the sample: 2**

Sample Preparation and pre-treatment (for Feed samples):

Detection limit: 1ppb

- Weigh 1 ± 0.05 grams of the homogenized feed sample into a 50 ml centrifuge tube. Add 4 ml of **0.3M NaOH solution**. Mix well then add 8 ml **Ethyl acetate**, mix for 5 mins, and then centrifuge at 4000 r/min at room temperature (RT) for 10 mins.
- Take 1 ml supernatant in a new 10 ml glass tube and dry by nitrogen evaporator or water bath at 50 – 60 ^{0}C.
- After drying, dissolve the sample residue in 1 ml n-Hexane and then in 1 ml **Reconstitution Buffer (1X)**. Mix well for 2 mins. Centrifuge at 4000 r/min at RT for 5 mins.
- Remove the upper layer. Take 100 µl of the lower liquid for the analysis
- **Fold of dilution of the sample: 8**

Sample Preparation and pre-treatment (for Milk sample):

Detection limit: 0.5ppb

- Take 200 µl of the milk sample and add 0.8 ml **Reconstitution Buffer (1X)**. Mix thoroughly.
- Take 100 µl for the analysis
- **Fold of dilution of the sample: 5**

VIII. Assay Protocol:

Note: Bring all reagents and samples to room temperature 30 minutes prior to the assay.
It is recommended that all standards and samples be run at least in duplicate.
A standard curve must be run with each assay.

1. Add 100 µl of the **sample or standards** to separate duplicate wells and then add 50 µl **Antibody working solution** into each well. Mix gently by shaking the plate manually, seal the microplate with the plate sealer, and incubate at 25 ^{0}C for 30 minutes in dark.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 300 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step five times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 100 µl **HRP Conjugate** to each well. Incubate at 25 ^{0}C for 30 minutes in dark.
4. Repeat washing as mentioned in **step 2**.
5. Add 50 µl of the **Substrate A** and then 50 µl of the **Substrate B** into each well. Mix gently for 5 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins at dark.
6. Add 50 µl of the **Stop Solution** into each well. Mix gently by shaking the plate manually. Set the wavelength of the microplate reader at 450 nm to determine the OD value (Recommend reading the OD value at the wavelength 450 nm within 5 mins).

IX. Calculation:

- **Quantitative determination**

The mean values of the absorbance values obtained for the standards and the samples are divided by the absorbance value of the first standard (zero standard) and multiplied by 100%. The zero standard is thus made equal to 100% and the absorbance values are quoted in percentages.

Absorbance Value (%) = B/B_0 X 100%

B: The average absorbance value of the sample or standard

B_0: The average absorbance value of the 0 ppb standard

To draw a standard curve: Take the absorbency value of standards as y-axis, logarithmic of the concentration of the Dexamethasone standards solution (ppb) as x-axis. The Dexamethasone concentration of each sample (ppb), which can be read from the calibration curve, is multiplied by the corresponding dilution factor of each sample followed, and the actual concentration of sample is obtained.

X. Related Products:

Sulfadiazine ELISA Kit (E4904)
Chloramphenicol (CAP) ELISA Kit (K4230)
Norfloxacin ELISA Kit (E4776)
Amoxicillin ELISA Kit (E4614)
Furazolidone ELISA Kit (K4231)