



User's Manual

Dexamethsaone ELISA Kit



DEIA7038



96T



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

Dexamethasone ELISA Test Kit is a competitive enzyme immunoassay for the detection of Dexamethasone, Betamethasone and Flumethasone in meat, liver, and milk.

The unique features of the kit are:

1. High recovery (>75%), rapid, and cost-effective extraction methods
2. High sensitivity (0.05 ng/g or ppb) and low detection limit (0.11 ng/g or ppb) for milk samples
3. Rapid ELISA assay (less than 2 hours regardless of number of samples)
4. High reproducibility

Principles of Testing

The test is based on a competitive colorimetric ELISA assay. The drug of interest has been coated in the plate wells. During analysis, sample is added along with the primary antibody specific for the target drug. 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

Dexamethasone ELISA Test Kit has the capacity for 96 determinations or testing of 42 samples in duplicate (assuming 12 wells for standards). Return any unused microwells to the foil bag and reseal them with the desiccant provided in the original package.

* If you are not planning to use the kit for over 1 month, Dexamethasone Antibody #1 and 100x HRP-Conjugated Antibody #2 at -20°C or in a freezer is recommended.

Kit Contents	Amount	Storage
Dexamethasone-Coated Plate	1 x 96-well plate (8 wells x 12 strips)	2-8°C
Dexamethasone Standards:		
Negative control	1.8mL	
0.05 ng/mL	1.8mL	
0.2ng/mL	1.8mL	
0.8ng/mL	1.8mL	
3.2ng/mL	1.8mL	
12.8 ng/mL	1.8mL	
100 ng/mL (spiking, optional)	1.8mL	
Dexamethasone Antibody #1	6mL	
HRP-Conjugated Antibody #2	12mL	
20× Wash Solution	30mL	
Stop Buffer	12mL	
TMB Substrate	12mL	
10X Sample Dilution Buffer	25mL	
Sample Extraction Buffer A	5 mL	
Sample Extraction Buffer B	5 mL	

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Incubator
3. Vortex mixer, (e.g. Gneie Vortex mixer from VWR)
4. 10, 20, 100 and 1000 µL pipettes
5. Multi-channel pipette: 50-300 µL (Optional)

Storage

Store the kit at 2-8°C. The shelf life is 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 (20-25°C / 68-77°F) or in a refrigerator before use.

Preparation of 1X Sample Dilution Buffer

Mix 1 volume of 10X Sample Dilution Buffer with 9 volumes of distilled water.

Preparation of 2X Sample Dilution Buffer

Mix 2 volume of 10X Sample Dilution Buffer with 8 volumes of distilled water.

Milk

1. Take 1 mL of milk sample, add 50µL of Sample Extraction Buffer A, vortex for 5 seconds, add 50µL of Sample Extraction Buffer B.
2. Vortex at maximum speed for 30 seconds.
3. Centrifuge at 4,000 × g for 5 minutes at room temperature .
4. Transfer 0.15mL of upper layer to a new tube, add 0.15µL of 2× Sample Dilution Buffer. Vortex at maximum speed for 30 seconds.
5. Use 100µL of the sample in the assay.

Note: Dilution factor: 2.2

Milk Powder

1. Weigh out 1g of milk powder sample, add 10mL of distilled water. Vortex for 5 minutes at maximum speed.
2. Take 1 mL of milk sample, add 50µL of Sample Extraction Buffer A, vortex for 5 seconds, add 50µL of Sample Extraction Buffer B.
3. Vortex at maximum speed for 30 seconds.
4. Centrifuge at 4,000 × g for 5 minutes at room temperature .
5. Transfer 0.15mL of upper layer to a new tube, add 0.15µL of 2× Sample Dilution Buffer. Vortex at maximum speed for 30 seconds.
6. Use 100µL of the sample in the assay.

Note: Dilution factor: 2.2

Meat/Liver

1. Weigh out 1g of the sample, add 1.9mL of Sample Balance Buffer 2× Sample Dilution Buffer. Mix well by vortex for 5 minutes at maximum speed. Add 50µL of Sample Extraction Buffer A, vortex for 5 seconds, add 50µL of Sample Extraction Buffer B.
2. Vortex at maximum speed for 30 seconds.
3. Centrifuge at 4,000 × g for 5 minutes at room temperature .
4. Use 100µL of the sample in the assay.

Note: Dilution factor: 3

Reagent Preparation

IMPORTANT: All reagents should be brought up to room temperature before use (1 - 2 hours at 20-25°C / 68-77°F); Make sure you read "Precautions" section. Solutions should be prepared just prior to ELISA test. 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.

Preparation of 1X Wash Solution

Mix 1 volume of the 20X Wash Solution with 19 volumes of distilled water.

Assay Procedure

Label the individual strips that will be used and aliquot reagents as the following example:

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

1. Add 100 µL of each Dexamethasone Standards and sample in duplicate into different sample wells.
2. Add 50 µL of Antibody #1 and mix well by gently rocking the plate manually for 1 minute.
3. Incubate the plate for 30 minutes at room temperature (20-25°C / 68-77°F).
4. 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).
5. Add 100 µL of 1X Antibody #2 solution and mix well by gently rocking the plate manually for 1 minute.
6. Incubate the plate for 30 minutes at room temperature (20-25°C / 68-77°F).
7. Wash the plate 3 times with 250 of µL 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 to each well. 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. Covering the microtiter plate while incubating is recommended).
9. After 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

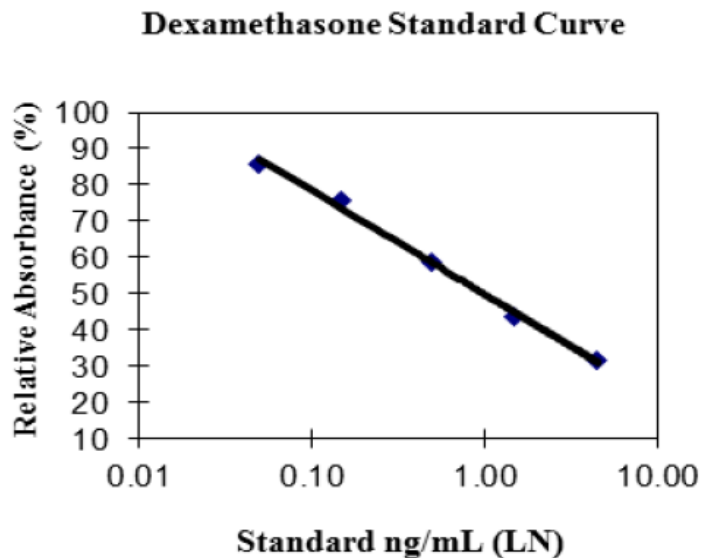
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a Logarithm curve.

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

When use computing software, recommends Log/Log standard curves.

Typical Standard Curve

The following figure is a typical Dexamethasone standard curve.



Detection Limit

Sample Type	Detection Limit (ng/g or ppb)
Milk	0.11
milk powder	0.11
Meat/Liver	0.15

Specificity

Analytes	Cross-Reactivity (%)
Dexamethasone	100
Betamethasone	50
Flumethasone	40
Prednisolone	50
Prednisone	10
Methylprednisolone	<1
Stanozolol	<0.1

Precautions

1. Do not use the kit past the expiration date.
2. Do not intermix reagents from different kits or lots except for components with the same part No's within their expiration dates. ANTIBODIES AND PLATES ARE KIT-AND LOT-SPECIFIC.
3. Make sure that the 100X HRP-Conjugated Antibody #2 and Antibody #2 Diluent are mixed in correct volumes.
4. Try to maintain a laboratory temperature of 20–25°C (68°–77°F). Avoid running assays under or near air vents, as this may cause excessive cooling, heating and/or evaporation. Also, do not run assays in direct

sunlight, as this may cause excessive heat and evaporation. Cold bench tops should be avoided by placing several layers of paper towel or some other insulation material under the assay plates during incubation.

5. Make sure you are using only distilled or deionized water since water quality is very important.
6. When pipetting samples or reagents into an empty microtiter plate, place the pipette tips in the lower corner of the well, making contact with the plastic.
7. Incubations of assay plates should be timed as precisely as possible. Be consistent when adding standards to the assay plate. Add your standards first and then your samples.
8. Add standards to plate only in the order from low concentration to high concentration as this will minimize the risk of compromising the standard curve.
9. Always refrigerate plates in sealed bags with a desiccant to maintain stability. Prevent condensation from forming on plates by allowing them equilibrate to room temperature (20-25°C / 68-77°F) while in the packaging.