Version 23-07/24

User's Manual

Prednisolone ELISA Kit

REF **DEIA-WZ117**

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.

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PRODUCT INFORMATION

Intended Use

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

General Description

The unique features of the kit are:

High recovery (>75%), rapid, and cost-effective extraction methods

High sensitivity (0.05 ng/g or ppb) and low detection limit (0.11 ng/g or ppb) for milk samples

Rapid ELISA assay (less than 2 hours regardless of number of samples)

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

Prednisolone 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.

1. Prednisolone-Coated Plate, 1 x 96-well plate (8wells x 12 strips)
2. Prednisolone Standards:Negative control, 0.05, 0.2, 0.8, 3.2, 12.8 ng/mL Standard, 100ng/mL(spiking, optional),1.8mL/vial
3. Prednisolone Antibody #1, 6mL
4. HRP-Conjugated Antibody #2, 12mL
5. 20× Wash Solution, 30mL
6. Stop Buffer, 12mL
7. TMB Substrate, 12mL
8. 10X Sample Dilution Buffer, 25mL
9. Sample Extraction Buffer A, 5 mL
10. Sample Extraction Buffer B, 5 mL

If you are not planning to use the kit for over 1 month ,Prednisolone Antibody #1 and HRP-Conjugated

Antibody #2 at -20°C or in a freezer is recommended.

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℉) 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 8,000 x g for 5 minutes at room temperature .
4. Transfer 150µL of upper layer to a new tube,add 150µL of 2x 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 8,000 x g for 5 minutes at room temperature.
5. Transfer 150µLof upper layer to a new tube,add 150µL of 2x 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 2x Sample Dilution.Buffer ,Mix well by vortex for 3 minutes at maximum speed or Mix in a multi-mixer for 20 minutes. Add 50µL of Sample Extraction Buffer A,vortex for 5 seconds, add 50µ L of Sample Extraction Buffer B.
2. Vortex at maximum speed for 30 seconds.
3. Centrifuge at 8,000 x g for 5 minutes at room temperature .
4. Use 100µ L 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
Prednisolone 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 Prednisolone 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℉).
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℉).
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℉), 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

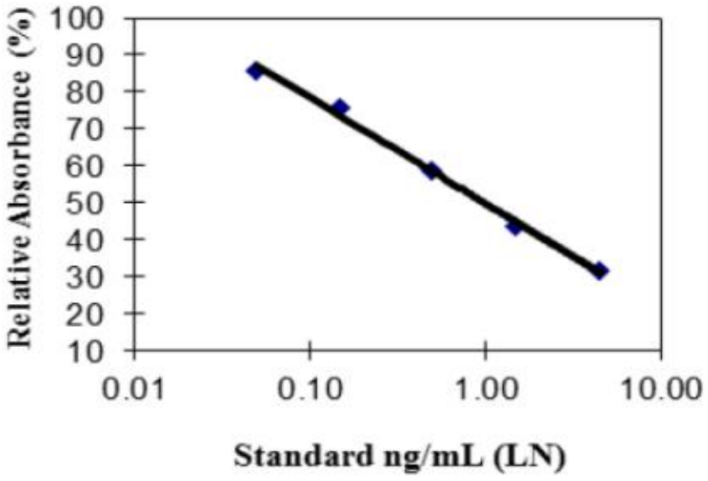
A standard curve can be constructed by plotting the mean relative absorbance (%) obtained from each reference standard against its concentration in ng/mL on a logarithmic curve.

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 standard curve.

Typical Standard Curve

The following figure is a typical Prednisolone standard curve.



Detection Limit

Milk: 0.11 ng/g or ppb

Milk powder: 0.11ng/g or ppb

Meat/Liver: 0.15 ng/g or ppb

Specificity

Prednisolone 100%

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. 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.
4. Make sure you are using only distilled or deionized water since water quality is very important.
5. 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.
6. 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.
7. 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.
8. 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℉) while in the packaging.