Version 13-08/24

User's Manual

Ribavirin ELISA Kit

REF **DEIANJ43**

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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

Ribavirin ELISA Test Kit enables government agencies, food manufacturers and quality assurance organizations to detect ribavirin in meat,egg,milk,serum to satisfy customer concerns about food safety.

The unique features of the kit are:

1. The extraction of ribavirin from samples can be finished in 30 minutes.
2. A quick ELISA assay (less than 3 hours regardless of number of samples).
3. High reproducibility.

Principles of Testing

This kit adopts the indirect competitive ELISA method, pre-coating the coupled antigen on the microwell strip, and the residual ribavirin in the sample and the pre-coated coupling antigen on the microwell strip compete for the ribavirin antibody. After the enzyme is labeled, the color is developed with the TMB substrate, and the absorbance value of the sample is negatively correlated with the content of the residue ribavirin contained in it. Comparing with the standard curve, the content of the corresponding residue ribavirin can be obtained.

Reagents And Materials Provided

1. Ribavirin -Coated Plate: 1x96-well Plate (8wells x12strips)
2. Ribavirin Standards: 0.2ppb, 0.6ppb, 1.8ppb, 5.4ppb, 16.2ppb, 1.5mL for each.
3. Negative control: 1.5mL
4. 11X HRP-Conjugate: 0.8 mL
5. HRP-Conjugate Diluent: 8 mL
6. Sample Diluent: 20 mL
7. 20X Wash Solution: 25 mL
8. Stop Buffer: 7 mL
9. TMB Substrate: 14 mL

Materials Required But Not Supplied

1. Microtiter plate reader (450 nm)
2. Tissue Mixer (e.g. Omni TissueMaster Homogenizer)
3. Vortex mixer (e.g. Genie Vortex mixer from VWR)
4. 10, 20, 100 and 1000 uL pipettes
5. Multichannel pipette: 50-300 uL (Optional)

Storage

Ribavirin 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. Store the kit at 2-8C *. 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.

1. Preparation Sample Extraction Solution:

Accurately weigh out 5.0 g of trichloroacetic acid, add 500 mL of deionized water, and fully dissolve it.

2. Preparation 0.2 M $Na_2HPO_4 \cdot 12H_2O$:

Accurately weigh 5.73 g of disodium hydrogen phosphate dodecahydrate and add 80 mL of deionized water to fully dissolve it.

Meat (Chicken, Duck, Pork)

1. Accurately weigh 1±0.01 g homogenized sample into a 50 mL centrifuge tube;
2. Add 6 mL of Sample Extraction Solution and vortex for 1 min;
3. Above 4000 g, centrifuge for 5 min;
4. Take 500 µL of the supernatant in a new centrifuge tube, add 100 µL of 0.2 M $Na_2HPO_4 \cdot 12H_2O$, and vortex for 30 seconds;
5. Over 4000 g, centrifuge for 1 min;
6. Use 50 µL in the assay.

Note: Dilution factor: 7.

Milk

1. Take 1 mL of raw milk in a 50 mL centrifuge tube;
2. Add 2 mL of Sample Extraction Solution and vortex for 1 min;
3. Above 4000 g, centrifuge for 5 min;
4. Take 500 µL of the supernatant into a new centrifuge tube, add 120 µL 0.2 M $Na_2HPO_4 \cdot 12H_2O$, and vortex for 30 s;
5. Over 4000g, centrifuge for 1 min;
6. Use 50 µL in the assay.

Note: Dilution factor: 3.

Egg

1. Weigh 1±0.01 g of the homogenized whole egg sample into a 50 mL centrifuge tube;
2. Add 2 mL of Sample Extraction Solution and vortex for 1 min;
3. Above 4000 g, centrifuge for 5 min;
4. Take 500 µL of the supernatant into a new centrifuge tube, add 100 µL 0.2 M $Na_2HPO_4 \cdot 12H_2O$, and vortex for 30 s;

5. Above 4000 g, centrifuge for 1 min;
6. Take 100 µL of supernatant into a new centrifuge tube, add 200 µL of Sample Diluent, and vortex for 30 s;
7. Use 50 µL in the assay.

Note: Dilution factor: 9.

Serum

1. Take 50µL serum sample in a centrifuge tube;
2. Add 200µL Sample Diluent and vortex for 1 min;
3. Above 4000 g, centrifuge for 5 min;
4. Use 50 µL in the assay.

Note: Dilution factor: 5.

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.

1. Preparation of 1X Wash Solution

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

2. 1X HRP-Conjugate

Mix 1 volume of the 11X HRP-Conjugate with 10 volumes of HRP-Conjugate Diluent.

Assay Procedure

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

Component	Volume per Reaction	24 Reactions
1X HRP Conjugate	50 uL	1.2 mL
1X Wash Solution	2.0 mL	48 mL
Stop Buffer	50 uL	1.2 mL
TMB Substrate	100 uL	2.4 mL

1. Add 50 uL of each Standards in duplicate into different wells (Add standards to plate only in the order from low concentration to high concentration).
2. Add 50 uL of each sample in duplicate into different sample wells.
3. Add 50 uL of 1X HRP Conjugate and mix well by gently rocking the plate manually for 1 minute.
4. Incubate the plate for 30 min at room temperature (20 – 25°C / 68 – 77°F) in the dark.
5. Wash the plate 4 times with 250 uL 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 uL of TMB Substrate . Mix the solution by gently rocking the plate manually for 1 minute while incubating. Incubate for 15 minutes at room temperature (20 – 25°C / 68 – 77°F) in the dark. (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).
7. After incubation, add 50 uL of Stop Buffer to stop the enzyme reaction.
8. 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.

$$\text{Relative absorbance (\%)} = \frac{\text{absorbance standard (or sample) x 100}}{\text{absorbance zero standard}}$$

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. A special program with Excel functionality.

Sensitivity

Meat…… 8

Milk…… 4

Egg…… 2

Serum…… 1

Specificity

Ribavirin…… 100.0%

Precautions

1. The standards contain ribavirin. 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 their expiration dates. HRP CONJUGATES AND PLATES ARE KIT-AND LOT-SPECIFIC.
4. Try to maintain a laboratory temperature of 20°C–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 – 25C / 68 – 77F) while in the packaging.