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Ribavirin ELISA Kit

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(Catalog # E4949-100, 96 assays, Store at 4°C)

I. Introduction:

Ribavirin is a guanosine analog and an anti-viral agent that interferes with viral mRNA synthesis and capping. Upon activation by adenosine kinase, ribavirin triphosphate binds to the nucleotide-binding site of the viral mRNA polymerase, thereby preventing the binding of correct nucleotides and causing decreased viral replication and formation of defective virions. Ribavirin also inhibits viral mRNA guanylyltransferase and 2′-O-methyltransferase, thereby disrupting the posttranslational capping of the viral mRNA. The drug is recommended to treat hepatitis C and viral hemorrhagic fevers. BioVision's Ribavirin ELISA kit is used to quantitatively measure Ribavirin in muscle tissue, milk, and egg 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-Ribavirin 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 Ribavirin in the samples.

II. Application:

This ELISA kit is used for *in vitro* quantitative determination of Ribavirin
Detection Limit: 8ppb for muscle tissue, 2ppb for raw milk, 4ppb for egg
Sensitivity: 0.2ppb
Cross reaction: Ribavirin 100%

III. Sample Type:

Tissue, Milk, Egg

IV. Kit Contents:

Components	E4949-100	Part No.
Micro ELISA Plate	8 X 12 Strips	E4949-100-1
Standard (S0 – S6)	1 ml X 6	E4949-100-2
HRP Conjugate (11X)	0.8 ml	E4949-100-3
HRP Conjugate diluent	8 ml	E4949-100-4
Substrate A	6 ml	E4949-100-5
Substrate B	6 ml	E4949-100-6
Stop Solution	6 ml	E4949-100-7
Wash Buffer (20X)	25 ml	E4949-100-8
Sample diluent	20 ml	E4949-100-9
Plate Sealer	3	E4949-100-10

V. User Supplied Reagents and Equipment:

- Chemicals: deionized water, Trichloroacetic acid, $NaHPO_4.12H_2O$
- 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:** To prepare 1X Wash Buffer, dilute 1 part of Wash buffer (20X) with 19 parts of deionized water. Prepare quantity as needed.

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

Standards	S0	S1	S2	S3	S4	S5
Conc. (ppb)	0	0.2	0.6	1.8	5.4	16.2

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.

FOR RESEARCH USE ONLY! Not to be used on humans.

Solution preparation before sample pre-treatment:

1) **Sample extracting solution:** Weigh 4 grams of **Trichloroacetic acid** and dissolve it in 50 ml deionized water. Mix well.
2) **0.2M $NaHPO_4.12H_2O$ solution:** Weigh 5.73 grams of **$NaHPO_4.12H_2O$** and dissolve it in 80 ml with deionized water. Mix well
3) **HRP Conjugate solution (1X):** Dilute 1 part of **HRP Conjugate (11X)** with 10 parts of HRP Conjugate diluent. **Do not store this solution, use it immediately for the experiment.**

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

Detection limit: 8ppb

- Take 1 ± 0.01 grams of the homogenized tissue sample into a 50 ml centrifuge tube. Add 6 ml of **Sample extracting solution**. Mix for 1 min. Centrifuge at 4000 r/min for 5 mins.
- Take 500 µl of the supernatant in a new tube and add 100 µl of **0.2M $NaHPO_4.12H_2O$ solution**. Mix for 30 secs. Centrifuge at 4000 r/min for 1 min.
- Take 50 µl of the supernatant for the analysis.
- **Fold of dilution of the sample: 7**

Sample Preparation and pre-treatment (for raw milk samples):

Detection limit: 2ppb

- Take 1 ml of the raw milk in a centrifuge tube and add 2 ml of **Sample extracting solution**. Mix for 1 min. Centrifuge at 4000 r/min for 5 mins.
- Take 500 µl of the supernatant in a new tube; add 120 µl of **0.2M $NaHPO_4.12H_2O$ solution**. Mix for 30 secs. Centrifuge at 4000 r/min for 1 min.
- Take 50 µl of this mixture for the analysis.
- **Fold of dilution of the sample: 3**

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

Detection limit: 4ppb

- Take 1 ± 0.01 grams of the homogenized egg sample into a 50 ml centrifuge tube. Add 2 ml of **Sample extracting solution**. Mix for 1 min. Centrifuge at 4000 r/min for 5 mins.
- Take 500 µl of the supernatant in a new tube and add 100 µl of **0.2M $NaHPO_4.12H_2O$ solution**. Mix for 30 secs. Centrifuge at 4000 r/min for 1 min.
- Take 100 µl of the supernatant in a new tube; add 200 µl of the **Sample diluent**. Mix for 30 secs.
- Take 50 µl of this mixture for the analysis.
- **Fold of dilution of the sample: 9**

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 50 µl of the **sample or standards** to separate duplicate wells. Add 50 µl of the **HRP conjugate (1X)** into each well. Mix gently for 10 secs by shaking the plate manually, seal the microplate with the plate sealer, and incubate in dark at 25 ^{0}C for 30 minutes.
2. Remove the plate sealer carefully, aspirate liquid out of microwells, and add 260 µl of **Wash Buffer (1X)** to each well. Wash for 30 secs, and then discard the buffer. Repeat the washing step four times. After the final wash step, tap to dry (if there are the bubbles after tapping, remove them with the clean tips).
3. Add 50 µl of the **Substrate A** and then add 50 µl of the **Substrate B** into each well. Mix gently for 15 secs by shaking the plate manually, and incubate at 25 ^{0}C for 15 mins in dark.
4. Add 50 µl of the **Stop Solution** into each well. Mix gently for 10 secs 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: Plot the absorbance value of standards as y-axis, logarithmic of the concentration of the Ribavirin standards solution (ppb) as x-axis. The Ribavirin 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:

Sulfamethazine ELISA Kit (E4778)
Norfloxacin ELISA Kit (E4776)
Chlortetracycline ELISA Kit (E4782)
Ampicillin ELISA Kit (E4350)
Salbutamol (SALB) ELISA Kit (K4209)