Product No. A-3177
Lot 126H8865

Acyclovir-Horseradish Peroxidase Conjugate

For Immunoassay

Acyclovir (acycloguanosine, (9-[(2-hydroxyethoxy)-methyl]-guanine, Zovirax®) conjugated to horseradish peroxidase (Acyclovir-HRP) for use in immunoassay is supplied ready-to-use for 100 double antibody enzyme immunoassay (EIA) tests when used with Rabbit anti-Acyclovir antiserum (Sigma Product No. A-3427). Acyclovir-HRP is provided as a liquid in 0.1 M phosphate buffer, pH 7.4, containing 1 mM EDTA, 200 µg/ml rabbit IgG, 10% bovine calf serum and 0.01% thimerosal as a preservative.

The Acyclovir-HRP EIA is a competitive binding immunoassay in which Acyclovir-HRP and unlabeled Acyclovir (standard or unknown sample) compete for a limited number of combining sites present in the rabbit antiserum to Acyclovir. Separation of the bound and free Acyclovir-HRP is accomplished using a specific immunoprecipitation reagent containing goat anti-rabbit IgG antiserum. The ratio of Acyclovir-HRP conjugate bound in the presence of Acyclovir to that bound without Acyclovir is inversely proportional to the concentration of Acyclovir (see sample data).

The Acyclovir-HRP EIA procedure described in this product insert will allow the determination of as little as 0.05 ng of Acyclovir per assay tube.

Lot Specific Data

Acyclovir-HRP	55.5 ng/ml
Volume per bottle	10 ml

Sample Data

Below is an example of a typical antigen addition curve generated using the reagents and methods described in this data sheet.

ng/ml	Absorbance	%B/Bo	Intercepts
0.0	0.833	100.0	
0.5	0.752	90.3	90% 0.5 ng/ml
2.5	0.679	81.5	50% 24.5 ng/ml
10.0	0.517	62.1	25% 174.0 ng/ml
40.2	0.358	43.0	
160.5	0.216	25.9	

Cross-Reactivity

The specificity of the Acyclovir-HRP EIA was determined by calculating the ratio of the moles of Acyclovir to moles of Acyclovir analog at the 50% intercept of the dose response curve and multiplying the result by 100%.

Analog	%
Guanine	0.090
Guanosine	0.011
Cyclic guanosine monophosphate	0.015
Ganciclovir	6.797
Dideoxyinosine	0.012
Adenosine	0.003

Recommended EIA Procedure

Reagents Required

	Sigma Product No.
Acyclovir-HRP	A-3177
Rabbit anti-Acylovir antiserum	A-3427
Rabbit IgG Immunoprecipitation Reagent	R-8633
Acyclovir (acycloguanosine)	A-4669

Preparation of Reagents

1. Acyclovir-HRP

Carefully remove and discard the metal seal on the Acyclovir-HRP bottle. Firmly replace bottle cap and store Acyclovir-HRP at 4°C fro a maximum of one week. For extended storage, freeze in working aliquots at –20°C Under proper storage conditions, the Acyclovir-HRP will be stable for 1 year.

2. EIA Assay Buffer

Recommended buffer composition is: 0.1 M phosphate, pH 7.4 containing 1% bovine calf serum and 0.01% thimerosal as a preservative.

Prepare EIA assay buffer by dissolving the contents of 1 bottle Gal-Pac® (Sigma Product No. 936-4GP) in approxi-

mately 3 liters deionized water. Add 0.38 g thimerosal (Sigma Product No. T-5125) and 38 ml bovine calf serum (Sigma Product No. C-3284). Stir until all components are completely dissolved and bring the volume to 3.8 liters with deionized water. Store EIA assay buffer at 4°C.

3. Acyclovir Standards

Standards should be prepared in a matrix equivalent to the unknown samples. The range of the standards, after appropriate pre-dilution, is recommended to be 0.5 - 160 ng/ml for the procedure described below. Acyclovir (acycloguanosine, A-4669) is soluble at 1 mg/ml in EIA assay buffer or water (the solution may have to be brought to 37°C to completely dissolve the Acyclovir). Acyclovir standards are stable at 4°C with a preservative such as 0.01% thimerosal.

NOTE:Sodium azide interferes with horseradish peroxidase and should not be used.

4. Rabbit Anti-Acyclovir Antiserum

Reconstitute the Anti-Acyclovir antiserum with 10 ml EIA assay buffer to obtain a 10x stock antiserum solution. The 10x stock antiserum can be aliquoted into convenient volumes and stored frozen. To prepare assay strength Acyclovir antiserum, further dilute 1 part of 10x stock antiserum with 9 parts EIA assay buffer. Store assay strength antiserum at 4°C.

5. Rabbit IgG Immunoprecipitation Reagent

Rabbit IgG Immunoprecipitation Reagent is supplied as a ready to use mixture of goat anti-rabbit IgG antiserum in 0.1 M phosphate buffer, pH 7.4, containing 5 mM EDTA, 3.9% polyethylene glycol and 0.01% thimerosal as a preservative. Before use, gently mix by inversion. Store the immunoprecipitation reagent at 4°C.

6. Horseradish Peroxidase Substrate

The Acyclovir-HRP EIA described in this product insert uses hydrogen peroxide as the substrate and *o*-phenylenediamine dihydrochloride (OPD) as the hydrogen donor. The OPD forms a colored oxidation product that is measured at 490 nm after acidification. The absorbance of the reaction mixture is proportional to the Acyclovir-HRP concentration in the assay tube.

Substrate composition:

OPD (0.4mg/ml) and H_2O_2 (0.12%) in Phosphate-Citrate Buffer, pH 5.0.
Phosphate:Citrate Buffer (available as a tablet, Sigma Product No. P-4809)

Procedure

1. Label 12 x 75 mm test tubes with the appropriate standard or test sample identification.
2. Pipet 100 µl of Acyclovir-HRP to all test tubes.
   Optional: Add 100 µl Acyclovir-HRP to nonspecific binding tubes (NSB).
3. Pipet 100 µl standard or test sample to the appropriate test tube.
   Optional: Add 100 µl assay buffer to the NSB tubes.
4. Pipet 100 µl antibody to all tubes (except optional NSB tube).
   Optional: Add 100 µl assay buffer to NSB tubes.
5. Vortex gently in ensure complete mixing and incubate at ambient temperature for 1 hr. protected from light.
6. Pipet 1 ml Rabbit IgG Immunoprecipitation Reagent to all tubes.
7. Centrifuge at 2,000xg for 15 minutes at 4°C.
8. Aspirate the supernatant from each tube.
9. Disrupt the precipitate with brief, vigorous vortexing with the tube held vertically to reduce the amount of immune precipitate on the sides of the tubes above the 300 µl level.
10. Add 300 µl of freshly prepared substrate solution to each tube. Note the order and timing of the substrate addition.
11. Develop color with occasional agitation at ambient temperature for 30 minutes. Protected from light.
12. In the same order and with similar timing of substrate addition, add 750 µl 1 M sulfuric acid to all tubes.
13. Measure absorbance at 490 nm for each tube in an appropriate spectrophotometer, reduced the data and calculate results as appropriate.

Storage

The conjugate should be stored frozen in working aliquots. Repeated freezing and thawing is **not** recommended. Storage in "frost-free" freezers is **not** recommended. For continuous or daily use, the conjugate may be stored at 2-8°C.

References

Schaeffer, H.J., et al., Nature, **272**, 583 (1978).
Quinn, R.P., et al, Analytical Biochemistry, **98**, 319 (1979).