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ProductInformation

3N-AZIDO-3N-DEOXYTHYMIDINE-METHYL-^{3}H

Product No. **A 7803**

Product Description
Tritiated (^{3}H) 3N-Azido-3N-deoxythymidine (azidothymidine, AZT, zidovudine) for use in immunoassay is supplied ready to use for 100 double antibody radioimmunoassay (RIA) tests when used with Sigma AZT antiserum (Product No. A 9425).

The AZT-^{3}H RIA is a competitive binding immunoassay in which AZT-^{3}H and unlabeled AZT (standard or unknown sample) compete for a limited number of combining sites present in the rabbit antiserum to AZT. Separation of the bound and free AZT-^{3}H is accomplished using a specific immunoprecipitation reagent containing goat antiserum to rabbit IgG. The ratio of radioactivity bound in the presence of AZT to that bound without AZT is inversely proportional to the concentration of AZT (See sample data).

The AZT-^{3}H RIA procedure described in this product insert will allow the determination of as little as 0.1 ng of AZT per assay tube. The cross reactivity with AZT-glucuronide, the major metabolite of AZT, is 0.065%.

Specific Radiochemical Data

Activity per ml	0.07 ±0.005 µCi/ml

Reagents
Diluent: 0.1 M phosphate buffer, pH 7.4, containing 200 µg/ml rabbit IgG, 100 µg/ml bovine gamma globulins and 0.1% sodium azide as a preservative.

Precautions and Disclaimer
Due to sodium azide content a material safety data sheet (MSDS) for this product has been sent to the safety officer of your institution. Consult the MSDS for information regarding hazards and safe handling practices.

Recommended Radioimmunoassay Procedure
Required Reagents and Preparation

AZT-^{3}H (Product No. A 7803)
Store AZT-^{3}H at 4 °C. Under proper storage conditions, the AZT-^{3}H will be stable for 1 year.

RIA Assay Buffer
The RIA assay buffer consists of 0.1 M phosphate, pH 7.4, containing 0.1% sodium azide and 0.01% bovine gamma globulins and is available from Sigma as FPIA Dilution Buffer (Product No. F 3263).

RIA assay buffer may be prepared by dissolving the contents of 1 bottle of Gal-Pac 7 phosphate buffer (Product No. 936-4GP) in approximately 3 liters deionized water. Add 3.8 g sodium azide (Product No. S 2002), 0.38 g bovine gamma globulins (Product No. G 5009) and stir until all components are completely dissolved. Bring the volume to 3.8 liters with deionized water. Store RIA assay buffer at 4 °C.

Azidothymidine 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 1.5 - 60 ng/ml for the procedure described below. Azidothymidine (A2169) is readily soluble in RIA assay buffer. AZT standards are stable at 4 °C with a preservative such as 0.1% sodium azide.

Antiserum to AZT (Product No. A 9425)
Reconstitute the AZT antiserum with 10 ml RIA assay buffer to obtain working strength antiserum. Store reconstituted antiserum at 4 °C.

Rabbit IgG Immunoprecipitation Reagent
(Product No. R 8633)
Rabbit IgG Immunoprecipitation Reagent is supplied as a ready to use mixture of goat antiserum to rabbit IgG 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.

0.1 N Hydrochloric Acid (HCl)
0.1 N HCl is used to dissolve the precipitated immune complex prior to sampling for counting in a liquid scintillation counter. To prepare 0.1 N HCl combine 1 part 1 N HCl (Product Code 920-1) with 9 parts deionized water. The 0.1 N HCl can be stored at room temperature.

Liquid Scintillation Solution
Liquid scintillation solution is not supplied by Sigma. Any counting solution that has a high capacity for aqueous samples will be satisfactory.

Procedure

1. Label 12 x 75 mm test tubes with the appropriate standard or test sample identification.
2. Pipet 100 µl standard or test sample to the appropriate test tube.
   Optional: Add 100 µl standard 0 to the non-specific binding tubes (NSB) tubes.
3. Pipet 100 µl of AZT-^{3}H to all test tubes.
   Optional: Add 100 µl AZT-^{3}H to total radioactivity tubes (TR) and NSB.
4. Pipet 100 µl antiserum to all tubes (except optional TR and NSB tubes).
   Optional: Add 100 µl RIA assay buffer to NSB tubes.
5. Vortex gently to ensure complete mixing and incubate at ambient temperature for 1 hour.
6. Pipet 1 ml Rabbit IgG Immunoprecipitation Reagent to all tubes (except optional TR tubes).
7. Centrifuge at 2,000xg for 15 minutes at 4 °C.
8. Aspirate the supernatant from each tube (except optional TR tube).
9. Pipet 600 µl 0.1 N HCl to each tube (except optional TR tube) and mix well.
   Optional: Add 500 µl 0.1 N HCl to TR tube.
10. Transfer 500 µl of the contents of each tube into liquid scintillation vials and add liquid scintillation solution.
11. Count each sample for a minimum of 2 minutes, reduce data and calculate results as appropriate.

Sample Data
The following is an example of a typical antigen addition curve generated using the reagents and methods described in this product insert.

AZT-^{3}H (Product No. A 7803)
AZT antiserum (Product No. A 9425)
AZT standards prepared in bovine calf serum

Sample size: 100 µl per assay tube
Liquid Scintillation Counter
Counting Efficiency = 40%
Total Radioactivity (TR) = 11,384 DPM
%Bo/TR = 62.7%
Non-specific binding (NSB) = 297 DPM
%NSB/TR = 2.6%

ng/ml	DPM	%B/B$_0$		
0.0	7246	100.0		
1.5	6502	89.3		
4.0	5522	75.2	Intercepts	
10.0	3850	51.1	90%	1.4 ng/ml
25.0	2308	28.9	50%	10.5 ng/ml
60.0	1270	14.0	20%	336.3 ng/ml

Cross Reactivity
The specificity of the AZT-^{3}H RIA was determined by calculating the ratio of the moles of AZT to moles of AZT analogue at the 50% intercept of the dose response curve and multiplying the result by 100%.

Analogue	**%**
Thymidine	0.003
AZT-glucuronide	0.065
β-Thymidine	< 0.001
Dideoxythymidine	0.295
3-Methylthymidine	0.034
5N-Deoxythymidine	0.002

References
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Granich, G.G., et al., Antimicrobial Agents and Chemotherapy **33**; 1275 (1989).
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