



**ANA HUMAN REFERENCE SERUM #4**

Product Package Insert      Catalogue #IS2075      Lot 95-0055L      8/30/96 (rev. 1/05)

**ARTHRITIS FOUNDATION - CENTERS FOR DISEASE CONTROL REFERENCE SERUM FOR  
HUMAN ANTIBODIES TO NUCLEAR RIBONUCLEOPROTEIN (U-1 RNP)**

Intended Use

For in vitro immunodiagnostic use as a reference human serum having high levels of antibodies to nuclear RNP (also known as U-1 RNP and RNase-sensitive extractable nuclear antigen [ENA]) to be used to confirm the specificity of local standards.

Description of Reference Serum

Citrated plasma from a single donor was made 0.01 M CaCl<sub>2</sub> and 0.013 M epsilon-amino-n-caproic acid, allowed to clot at 4°C overnight, centrifuged, and frozen at -70°C until lyophilization. Volumes of 0.50 ml were dispensed into borosilicate vaccine vials, freeze-dried, and sealed with butyl rubber stoppers while still under reduced pressure. Vials are stored at the CDC at -20°C. The mean dry weight and SD of material in 10 randomly selected vials after freeze-drying was 21.3 ± 0.8 mg. The residual moisture content after freeze-drying was less than 1.0%.

The vial contents were sterile as determined by U.S.P. approved methods of sterility testing, negative for hepatitis-B surface antigen by radioimmunoassay, negative for antibody to HIV by Western blot, and free of rheumatoid factors as determined by latex agglutination and radioimmunoassay.

Reconstitution and Storage

Store the freeze-dried material at -20°C until use. To reconstitute, the contents should first be shaken to the bottom by tapping the upper end. Before the stopper is removed, the vacuum should be broken by insertion of a hypodermic needle through the rubber stopper. Precisely 0.50 ml of distilled water should then be added, and the vial restoppered. The freeze-dried powder should dissolve readily with gentle swirling (avoid foam). Allow to stand for at least 1 h before use and store at 4°C until use, not later than 24 h after reconstitution. Although not recommended, the reconstituted material will withstand at least 8 weekly freeze-thaws without loss of activity. If future use of reconstituted material is contemplated, portions of the undiluted material sufficient for a single use should be stored at -70°C and discarded after use.

α-U-1 RNP Antibody Content

This serum reacts strongly with nuclear ribonucleoprotein (U1-RNP). This antigen is usually present in rabbit and calf thymus. Thirteen reference laboratories used immunodiffusion techniques and several also used counterimmunoelectrophoresis, hemagglutination, ELISA, Western blot, or immunoprecipitation. All methods confirm the specificity and high titer of this serum for α-nuclear RNP. The median titer in immunodiffusion was 1:32 with a modified range of 1:8 to 1:64.

### Suggested Procedure for Standardization of Quality Control Reagents

Since the amount of AF/CDC reference preparation is limited, it should be used to calibrate secondary standards which can be run each day along with other samples being analyzed. The validity of any secondary standard depends on its having the same specificity as the primary AF/CDC standard (anti-nDNA, anti-Sm, etc.). The specificity can be determined by EIA, Western blot, double immunodiffusion (anti-nRNP, anti-Sm, anti-SSB/La) or by analysis for anti-nDNA (Farr assay, millipore filter assay, Crithidia test).

To confirm the specificity of a secondary standard in double diffusion or counterimmuno-electrophoresis:

1. Reconstitute the ampule as described above.
2. Use both undiluted (neat) and 1:4 dilutions of the AF-CDC sera and the secondary standard.
3. Arrange antibody wells such that the secondary standards (neat and 1:4) are each next to an undiluted and 1:4 dilution of the AF-CDC serum.
4. Run the routine test procedure using nuclear extract antigen.
5. Lines of identity should be obtained without extra lines or ambiguous reactions.

To calibrate a secondary standard (double diffusion, counterimmunoelectrophoresis):

1. Reconstitute the ampule as described above.
2. Prepare undiluted and serial four-fold dilutions up to 1:256 of the secondary standard and AF-CDC serum.
3. Run the routine test on all dilutions.
4. Relative potency =  $\frac{\text{reciprocal of end point dilution of secondary standard}}{\text{reciprocal of end point dilution of AF-CDC serum}}$

If the secondary standard is stable, the relative potency obtained should not change when periodically recalibrated. Any changes in day to day results with the secondary standard are more likely due to changes in test performance. Titer results may be particularly useful in assessing lot to lot changes in antigen preparations and for comparison of your results with the laboratories which initially evaluated these sera (see antibody content above).

### Caution

This serum was found to be negative for hepatitis-B surface antigen, hepatitis C and HIV antibody. Since no test method can offer complete assurance that these or other infectious agents are absent, this serum should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen (Centers for Disease Control, National Institutes of Health. Biosafety in Microbiological and Biomedical Laboratories, 1<sup>st</sup> Edition, 1984, 11-13.)

### Supplementary Information

The references for a general description of the AF-CDC reference sera are: Tan EM, et al. Reference Sera for Antinuclear Antibodies I. Arthritis Rheum 25:1003-1005,1982. Smolen JS et al. Reference Sera for Antinuclear Antibodies II. Arthritis Rheum 40: 413-418,1997. Detailed descriptions of the preparation, analysis, reagents, antigens, substrates, and individual reference laboratory results are in a supplementary publication available upon request:

AF-CDC ANA Reference Lab  
Immunology Branch, 17-3040, A25  
Centers for Disease Control and Prevention (CDC)  
Atlanta, GA 30333