

ANTISERUM to CORTISOL-3-CMO-BSA

CONTENTS:

1.0 ml antiserum diluted 1:125 in 0.05 M phosphate buffer, pH 7.5, with 0.1% human serum albumin and 0.05% NaN₃, lyophilized and sealed under nitrogen. This amount of antiserum is sufficient for 1 000 RIA-tubes. Reconstitute content in 1.0 ml distilled water.

IMMUNIZATION PROTOCOL:

Animal: Rabbit.

Immunogen: Cortisol-3-(0-carboxymethyl)-oxime was prepared according to Dean, P.D.G., Exley, D. and Johnson, M.W. (Steroides 18:593, 1971). Cortisol-3-(0-carboxymethyl)-oxime was conjugated to bovine serum albumin (Armour Pharmaceutical Company, Eastbourne, England) using isobutyl chloroformate, " the mixed anhydride method " (J. Biol. Chem. 228:713, 1957) - approx. 16 mol cortisol/mol albumin.

Immunization technique: Freund complete adjuvant. Multiple sites intradermal injections.

Priming dose: 600 ug of total conjugate.

Booster dose: 100 ug of total conjugate.

Number of booster injections: 1.

Booster interval: 13 weeks.

Sample collected: 2 weeks after booster injection.

ANTISERUM CHARACTERISTICS:

Assay method: Diluent for all reagents: 0.01 M phosphate buffer, pH 7.0, containing 0.9 NaCl, 0.2% EDTA, 0.1% gelatin and 0.02% Merthiolate. 100 ul of cortisol standard or sample, 200 ul cortisol-3-CMO-histamin-125I (~3 pg) and 200 ul antiserum (working dilution 1:25 000) were incubated for 18 hours at +4°C. Control tubes for non-specific binding and total activity were included. Bound and free ligand were separated by activated charcoal adsorption. 500 ul activated charcoal suspension (0.25% activated charcoal and 0.025% dextran T 70 in diluent) was added and the tubes were incubated for 10 minutes at +4°C before centrifugation. For details in methodology are referred to: Thorell J.I. and Larson S.M. Radioimmunoassay and related techniques, C.V.

Mosby, St. Louis 1978.

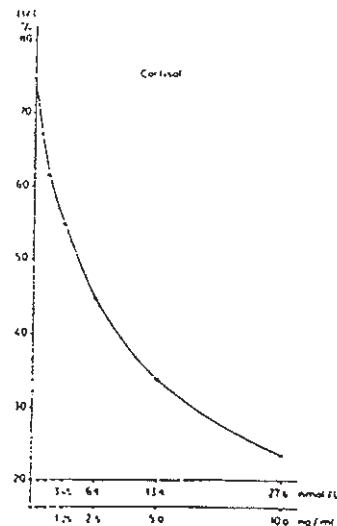
Titre: Final dilution in the assay is 1:62 500.

Avidity: The apparent association constant in the assay derived from a Scatchard plot is: $7 \times 10^9 \text{ L} \times \text{mol}^{-1}$. The half displacement concentration is 10.1-12.3 nmol/l (2.8-3.4-ng/ml) of the standard (= actual concentration of the sample).

Specificity: The antiserum shows the following cross-reactivity:

Steroid	Cross-reaction
Cortisol	100.0%
Cortisone	1.0%
11-deoxycortisol	8.9%
Prednisolone	31.6%
Cortisol-21-glucosiduronate	1.3%
Cortisol-21-sulfate	<1%
17 α -hydroxyprogesterone	<1%
Dexametazon	<1%
Prednison	<1%

In case of complaints or need for further information - please do not hesitate to contact us.



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