R&D® Ab
Research & Diagnostic Antibodies
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RIA Grade Anti-Human iNOS Serum (Rabbit)

AS-1640 **Lot # 8438**

This antiserum was raised in a rabbit which was immunized with synthetic human iNOS[1137-1153] covalently attached onto a carrier protein. The antiserum is specific for this peptide, cross reacts ~90% with intact hiNOS protein, and is suitable for the measurement of hiNOS by radioimmunoassay. Each bottle contains the appropriate amount of anti-hiNOS antiserum for 200 RIA tubes.

Antiserum Specificity

Polypeptide	% Cross Reactivity
hiNOS[1137-1153]	100
hiNOS	~90
hnNOS[1411-1433]	0
hnNOS	0
heNOS[1183-1203]	0
heNOS	0

Sample Preparation

Plasma: Draw blood into Na_2EDTA vacutainer tube (purple top tube) or a sterile syringe containing 1.5 mg of Na_2EDTA/ml blood to be drawn; place the vacutainer tube on ice or immediately transfer the blood to a chilled centrifuge tube on ice. When thoroughly cooled, centrifuge at 2500 x g for 30 min at 4^{o}C.

Tissue extracts: Tissues should be extracted by a procedure with will minimize proteolysis and maximize recovery. One such procedure is to homogenize in a cocktail of protease inhibitors and collect the supernatant by centrifugation.

Radioimmunoassay Procedure

To 12 x 75 mm tubes are added 0.10 ml of either buffer, standard or unknown and 0.10 ml of antiserum AS-1610 after rehydrating the lyophilized powder to 20 ml with assay buffer. The assays are incubated overnight at 4^{o}C before 0.10 ml of ^{125}I-hiNOS[Tyr^{1136}-1137-1153] which contains about 50,000 cpm is added or 0.10 ml of ^{125}I-hiNOS which contains about 100,000 cpm is added. The assays are incubated for an additional 24 hr before bound tracer is separated from free tracer. There are two recommended procedures for the separation of bound from free tracer: (1) by precipitation with goat anti-rabbit IgG antiserum and carrier normal rabbit IgG followed by centrifugation and aspiration or (2) by binding to mag-beads coated with goat anti-rabbit IgG antiserum followed by collection of the mag-beads on a magnet and decanting the supernatant. If possible, all dilutions and additions should be made in the RIA buffer: 0.1 M NaCl, 50 mM sodium phosphate, pH 7.2, 10 mM Na_2EDTA, 1.0% BSA, 0.1% Triton-X100, and 0.01% NaN_3 which contains 200 KIU aprotinin/ml.

Standard range: 1.0 – 128 fmole hiNOS[1137-1153] per tube

Maximum sensitivity: 4 fmole hiNOS[1137-1153] per tube