

**Certificate of Analysis**
**Product:** Affinity Purified Anti-Thyroid Hormone Receptor  $\alpha$  (THRA) [Rabbit] TRIAL SIZE

**Code:** 600-401-A38S

**Lot #** 20656

**Size:** 25  $\mu$ l

**Physical State:** Liquid (sterile filtered)

**Antibody Concentration:** 0.79 mg/ml (by UV absorbance at 280 nm)

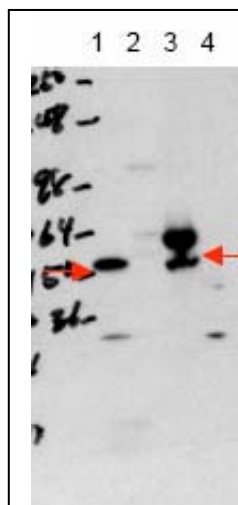
**Buffer:** 0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2

**Stabilizer:** None

**Preservative:** 0.01% (w/v) Sodium Azide

**Storage Conditions for Trial Size:** Store vial at  $-20^{\circ}$  C or below prior to opening. This vial contains a relatively low volume of reagent (25  $\mu$ l). To minimize loss of volume dilute 1:10 by adding 225  $\mu$ l of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge to collect the volume at the bottom of the vial. Use this intermediate dilution when calculating final dilutions as recommended below. Store the vial at  $-20^{\circ}$ C or below after dilution. Avoid cycles of freezing and thawing. Expiration date is three (3) months from date of opening.

**Background Information:** Thyroid hormone receptor alpha is a nuclear hormone receptor with high affinity for the hormone triiodo-thyronine. THRA is one of the several receptors for thyroid hormone, and has been shown to mediate the biological activities of thyroid hormone. Knockout studies in mice suggest that the different receptors, while having a certain extent of redundancy, may mediate different functions of thyroid hormone. THRA interacts with NCOA3 and NCOA6 co-activators, leading to a strong increase in transcription of target genes. THRA is localized within the nucleus and has been found to exist as 4 isoforms originating from alternative splicing variants. This antibody recognizes THRA isoform 1. Isoform 1 has a distinct C-terminus compared to isoform 2.



**Figure.** Western blot using Rockland's affinity purified anti-THRA antibody shows detection of purified recombinant THRA (lane 1) and THRA present in a 293 cell lysate after transient transfection with THRA (lane 3). No staining is evident in lysates from mock-transfected 293 cells (lane 2). Endogenous THRA is not detected in mouse brain whole cell lysate (lane 4). Nuclear extracts may be required to detect endogenous THRA as the protein localizes within the nucleus. The band at  $\sim$ 55 kDa, indicated by the arrowhead, corresponds to THRA. *Personal communication, S. Cheng and H. Ying, NCI, Bethesda, MD.*

**Application Note(s):** This affinity purified antibody has been tested for use in ELISA and western blotting. Specific conditions for reactivity should be optimized by the end user. Expect a band approximately  $\sim$ 55 kDa in size corresponding to THRA by western blotting in the appropriate cell lysate or extract. This antibody is capable of detecting transiently transfected THRA. Detection of endogenous THRA must be optimized by the end user.

**Recommended Dilutions:**

ELISA	1:650,000
WESTERN BLOT	1:1,000
IMMUNOPRECIPITATION	User Optimized
IF MICROSCOPY	User Optimized
OTHER APPLICATIONS	User Optimized

**Purity and Specificity:** This product was affinity purified from monospecific antiserum by immunoaffinity chromatography. This antibody reacts with human THRA protein. A BLAST analysis was used to suggest cross-reactivity with THRA from mouse, human and rat based on a 100% homology with the immunizing sequence. Cross-reactivity with THRA from other sources has not been determined.

**Immunogen:** This affinity purified antibody was prepared from whole rabbit serum produced by repeated immunizations with a synthetic peptide corresponding to a region near the amino terminus of human THRA isoform 1 protein.

**Relevant Links:** NCBI [NP\\_955366](#) Swiss-Prot [P10827-2](#)

#### Related Products:

<a href="#">#611-703-127</a>	Peroxidase Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (DONKEY) MX10
<a href="#">#611-132-122</a>	IRDye® 800 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#611-144-122</a>	DyLight™680 Conjugated Affinity Purified Anti-RABBIT IgG (H&L) (GOAT) MX10
<a href="#">#B501-0500</a>	BLOTTO (500 g)
<a href="#">#BSA-30</a>	30% BOVINE SERUM ALBUMIN SOL'N in 0.85% sodium chloride (no preservative or stabilizer) (500 ml)
<a href="#">#B304</a>	NORMAL GOAT SERUM (NGS) (10 ml)
<a href="#">#KIA-003</a>	<b>MaxTag™</b> Anti-RABBIT IgG Kit for Immunoblotting
<a href="#">#MB-070</a>	Blocking Buffer for Fluorescent Western Blotting

#### General References:

Ying,H., Araki,O., Furuya,F., Kato,Y. and Cheng,S.Y. (2007) Impaired adipogenesis caused by a mutated thyroid hormone alpha1 receptor. *Mol. Cell. Biol.* **27** (6), 2359-2371.

Nascimento,A.S., Dias,S.M., Nunes,F.M., Aparicio,R., Ambrosio,A.L., Bleicher,L., Figueira,A.C., Santos,M.A., de Oliveira Neto,M., Fischer,H., Togashi,M., Craievich,A.F., Garratt,R.C., Baxter,J.D., Webb,P. and Polikarpov,I. (2006) Structural rearrangements in the thyroid hormone receptor hinge domain and their putative role in the receptor function. *J. Mol. Biol.* **360** (3), 586-598.

Wan,W., Farboud,B. and Privalsky,M.L. (2005) Pituitary resistance to thyroid hormone syndrome is associated with T3 receptor mutants that selectively impair beta2 isoform function. *Mol. Endocrinol.* **19** (6), 1529-1542.

Laudet,V., Begue,A., Henry-Duthoit,C., Joubel,A., Martin,P., Stehelin,D. and Saule,S. (1991) Genomic organization of the human thyroid hormone receptor alpha (c-erbA-1) gene. *Nucleic Acids Res.* **19** (5), 1105-1112.

**Note:** This product is for research use only and is not intended for therapeutic or diagnostic applications. Please contact a technical service representative for more information. All products of animal origin manufactured by Rockland Immunochemicals are derived from starting materials of North American origin. Collection was performed in United States Department of Agriculture (USDA) inspected facilities and all materials have been inspected and certified to be free of disease and suitable for exportation. All properties listed are typical characteristics and are not specifications. All suggestions and data are offered in good faith but without guarantee as conditions and methods of use of our products are beyond our control. All claims must be made within 30 days following the date of delivery. The prospective user must determine the suitability of our materials before adopting them on a commercial scale. Suggested uses of our products are not recommendations to use our products in violation of any patent or as a license under any patent of Rockland Immunochemicals, Inc. If you require a commercial license to use this material and do not have one, then return this material, unopened to: Rockland Inc., P.O. BOX 326, Gilbertsville, Pennsylvania, USA.