

Datasheet for 100-1141**Aldolase Antibody****Overview**

Description:	Anti-Aldolase (Rabbit Muscle) (GOAT) Antibody - 100-1141
Item No.:	100-1141
Size:	2 mL
Applications:	IP, WB
Reactivity:	Human, Rabbit
Host Species:	Goat

Product Details

Background:	Aldolase plays a key role in glycolysis and gluconeogenesis. In addition, it may also function as scaffolding protein. In vertebrates, three forms of this ubiquitous glycolytic enzyme are found, aldolase A in muscle, aldolase B in the liver, and aldolase C in the brain. Alkylation of Arg-43 inactivates the enzyme. Aldolase is involved in step 4 of the subpathway that synthesizes D-glyceraldehyde 3-phosphate and glycero phosphate from D-glucose.
Synonyms:	goat anti-Aldolase Antibody, Fructose-bisphosphate aldolase A, Muscle-type aldolase
Host Species:	Goat
Clonality:	Polyclonal
Format:	Antiserum

Target Details

Gene Name:	ALDOA
Reactivity:	Human, Rabbit
Immunogen Type:	Native Protein
Immunogen:	Aldolase [Rabbit Muscle]

Purity/Specificity: This product was prepared from monospecific antiserum by a delipidation and defibrination. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-goat serum, purified and partially purified Aldolase [Rabbit Muscle]. This antibody will detect human Aldolase. Cross reactivity against Aldolase from other tissues and species may also occur. It has been reported that this antibody can detect human Aldolase on immunoblot showing a 41 kDa band in lysates from MCF7, NMB231 and HBL100 cell lines.

Relevant Links:

- [UniProtKB - P00883](#)
- [NCBI - NP_001075707.1](#)
- [GeneID - 100009055](#)

Application Details

Tested Applications: IP, WB

Application Note: Anti-Aldolase has been tested by immunoprecipitation and western blot and is suitable to be assayed against 1.0 µg of Aldolase [Rabbit Muscle] in a standard ELISA using Peroxidase conjugated Affinity Purified anti-Goat IgG [H&L] (Rabbit) code #605-4302 and (ABTS (2,2'-azino-bis-[3-ethylbenthiazoline-6-sulfonic acid])) code # ABTS-100 as a substrate for 30 minutes at room temperature. A working dilution of 1:3,000 to 1:12,000 of the reconstitution concentration is suggested for this product. Use approximately 5 ul of antibody to immunoprecipitate 50 ul of protein lysate.

Assay Dilutions: All assays should be optimized by the user. Recommended dilutions (if any) may be listed below.

ELISA: 1:5,000 - 1:20,000

IP: 1:100

WB: 1:500 - 1:5,000

Formulation

Physical State: Lyophilized

Concentration: 90 mg/mL by Refractometry

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

Preservative: 0.01% (w/v) Sodium Azide

Stabilizer: None

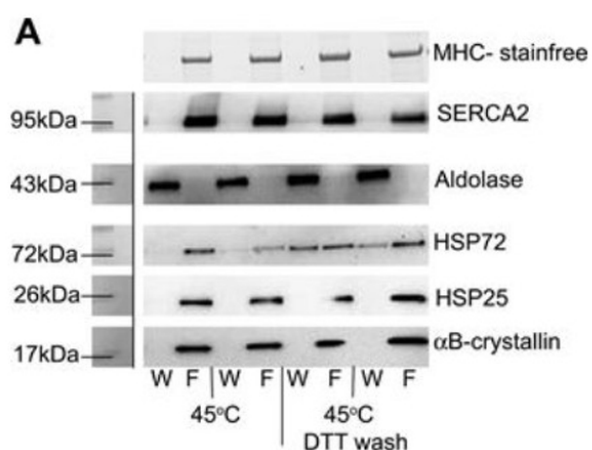
Reconstitution Volume: 2.0 mL

Reconstitution Buffer: Restore with deionized water (or equivalent)

Shipping & Handling

Shipping Condition:	Ambient
Storage Condition:	Store vial at 4° C prior to restoration. For extended storage aliquot contents and freeze at -20° C or below. Avoid cycles of freezing and thawing. Centrifuge product if not completely clear after standing at room temperature. This product is stable for several weeks at 4° C as an undiluted liquid. Dilute only prior to immediate use.
Expiration:	Expiration date is one (1) year from date of receipt.

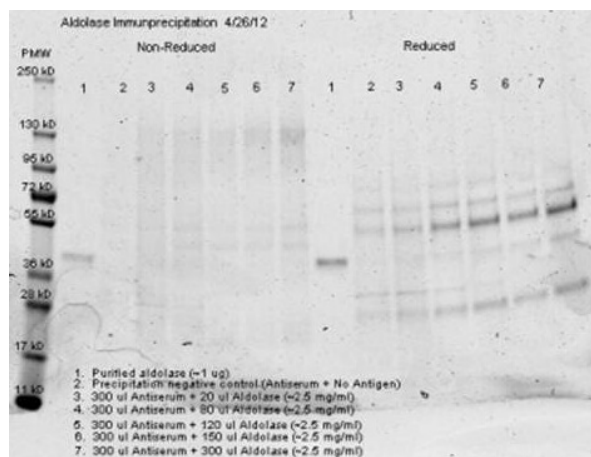
Images



Western Blot

Effect of DTT on HSP binding.

Dithiotreitol (DTT) reverses HSP72 binding in heat-treated fibers. A: Western blots showing HSPs remaining in skinned fiber (F) or diffusing into wash solution (W) when DTT (10 mM) was absent or present in wash solution (2 cases of each shown); skinned fibers obtained from SOL muscle were treated at 45°C for 30 min. HSP25 and αB-crystallin remained in the fiber irrespective of the presence of DTT, whereas HSP72 diffusion out of the fibers was enhanced in the presence of DTT. Aldolase remained freely diffusible in every fiber examined even with heat treatment to 45°C. Fig. 5. PMID: 22763123

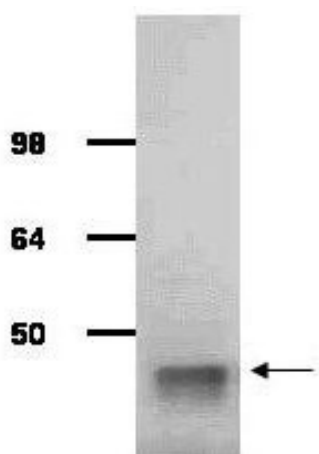


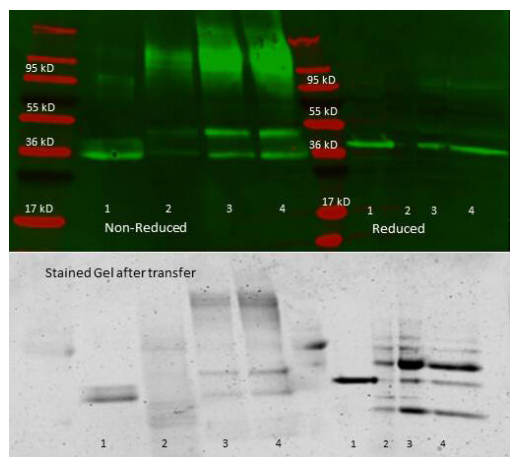
Immunoprecipitation

Immunoprecipitation of rabbit anti Aldolase antiserum – Immunoprecipitation performed with 300 ul of antiserum and an equal volume of varied amounts of purified aldolase diluted from a stock solution of ~2.5 mg/ml aldolase in PBS. Antibody/Antigen mixture was incubated ~24 hrs at 4°C, centrifuged for 6 minutes at 13K RPM, washed once with PBS, centrifuged and dissolved in 60 ul 0.1 N NaOH. 90 ul of PBS was added, the sample was divided in 2 portions, and an equal volume of reducing (+4% BME) or non-reducing 2X sample buffer was added. The reduced samples were boiled for five minutes, and all samples were run at 140 V for ~45 minutes on a 4-20% tris/glycine gradient gel. Gel was stained, destained and imaged (see attached) using standard protocols. Precipitation of aldolase was confirmed by comparison of increasing amounts of antigen with the control protein by SDS PAGE and observation of a 40-45 kD MW band corresponding to Aldolase. Additional higher and lower molecular weight bands correspond to serum proteins.

Western Blot

IgG purified antibody to rabbit muscle aldolase (100-1141, 200-1141 and 200-1341) was used at a 1:1000 dilution to detect human aldolase by Western blot. A whole cell lysate prepared from human derived A293 cells was loaded on a 4-12% tris glycine gradient gel for SDS-PAGE. The gel was transferred to nitro-cellulose using standard techniques. Antibody reaction with the membrane occurred overnight at 4° C in TTBS supplemented with 2% non-fat dry milk. Color was allowed to develop using SuperSignal West Pico Chemiluminescent Substrate (PIERCE). Other detection methods will yield similar results. This antibody clearly detects a band at ~41 kDa consistent with human aldolase.





Western Blot

Anti aldolase antibody – immunoprecipitation and western blot. 300 μ l aliquots of whole anti-aldolase antiserum (100-1141) were used to precipitate varying amounts of purified aldolase and precipitates with controls were compared by SDS-PAGE and Western blot. Samples shown in the image are: 1. Purified aldolase. 2. 300 μ l antiserum with no antigen (negative control). 3. 300 μ l antiserum with $\sim$ 100 μ l aldolase (2.5 mg/ml). 4. 300 μ l antiserum with $\sim$ 200 μ l aldolase (2.5 mg/ml). For the precipitation, 300 μ l of antiserum and an equal volume of aldolase antigen in PBS was incubated $\sim$ 24 hrs at 4°C, centrifuged for 6 minutes at 13K RPM, washed once with PBS, centrifuged and dissolved in 60 μ l 0.1 N NaOH. 90 μ l of PBS was added, the sample was divided in 2 portions, and an equal volume of reducing (+4% BME) or non-reducing 2X sample buffer was added. The reduced samples were boiled for five minutes, and all samples were run at 140 V for $\sim$ 45 minutes on a 4-20% tris/glycine gradient gel. Gel was stained, destained and imaged (see bottom image attached) using standard protocols. Precipitation of aldolase was confirmed by comparison of increasing amounts of antigen with the control protein by SDS PAGE and observation of a 40-45 kD MW band corresponding to Aldolase. Additional higher and lower molecular weight bands correspond to serum proteins.

References

- Ruethers T et al. Expanding the allergen repertoire of salmon and catfish. *Allergy*. (2021)
- Ruethers T et al. Variability of allergens in commercial fish extracts for skin prick testing. *Allergy*. (2019)
- Larkins NT et al. Influences of temperature, oxidative stress, and phosphorylation on binding of heat shock proteins in skeletal muscle fibers. *Am J of Physiology Cell Physiology* (2012)
- Janssen E et al. Impaired intracellular energetic communication in muscles from creatine kinase and adenylate kinase (M-CK/AK1) double knock-out mice. *J Biol Chem*. (2003)
- Ukaji F et al. Serum samples of patients with rheumatoid arthritis contain a specific autoantibody to “denatured” aldolase A in the osteoblast-like cell line, MG-63. *Ann Rheum Dis*. (1999)

Disclaimer

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