Datasheet for 200-1141S

Aldolase Antibody

Overview

Description:	Anti-Aldolase (Rabbit Muscle) (GOAT) Antibody - 200-1141S
Item No.:	200-1141S
Size:	25 µL
Applications:	ELISA, 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 glycerone phosphate from D-glucose.
Synonyms:	goat anti-Aldolase Antibody, Fructose-bisphosphate aldolase A, Muscle-type aldolase
Host Species:	Goat
Clonality:	Polyclonal
Format:	IgG

Target Details

Gene Name:	ALDOA
Reactivity:	Human, Rabbit
Immunogen Type:	Native Protein
Immunogen:	Aldolase [Rabbit Muscle]
Purity/Specificity:	Anti-ALDOLASE was prepared from monospecific antiserum by a delipidation, salt fractionation and ion exchange chromatography. Assay by immunoelectrophoresis resulted in a single precipitin arc against anti-Goat Serum, purified and partially purified Aldolase [Rabbit Muscle].

Relevant Links:	• UniProtKB - P00883 • NCBI - NP_001075707.1 • GeneID - 100009055

Application Details

Tested Applications:	ELISA, IP, WB
Application Note:	Anti-Aldolase Antibody has been tested by ELISA, immunoprecipitation, and western blot. This product is 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 1:10,000 to 1:40,000 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:	1:7,000
IP:	1:100
WB:	1:1,000 - 1:5,000

Formulation

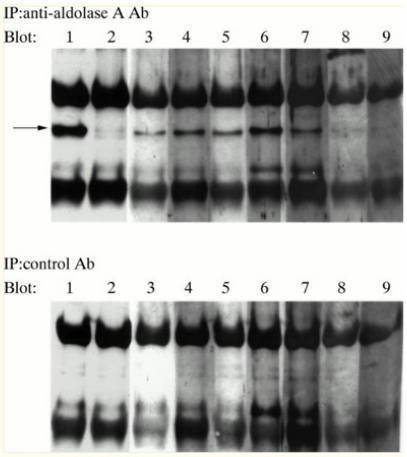
Physical State:	Liquid (sterile filtered)
Concentration:	1 mg/ml by UV absorbance at 280 nm
Buffer:	0.02 M Potassium Phosphate, 0.15 M Sodium Chloride, pH 7.2
Preservative:	0.01% (w/v) Sodium Azide
Stabilizer:	None

Shipping & Handling

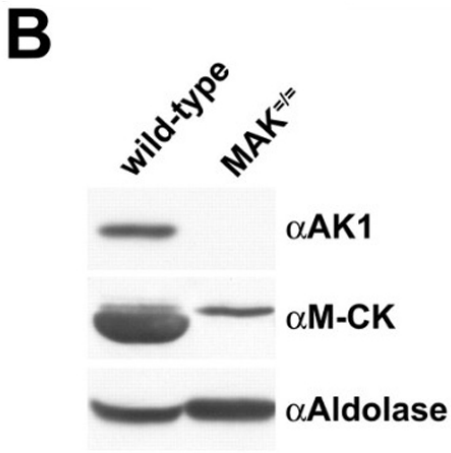
Shipping Condition:	Dry Ice
Storage Condition:	Store vial at -20° C or below prior to opening. This vial contains a relatively low volume of reagent (25 µL). To minimize loss of volume dilute 1:10 by adding 225 µL of the buffer stated above directly to the vial. Recap, mix thoroughly and briefly centrifuge 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°C or below after dilution. Avoid cycles of freezing and thawing.

Expiration: Expiration date is one (1) year from date of receipt.

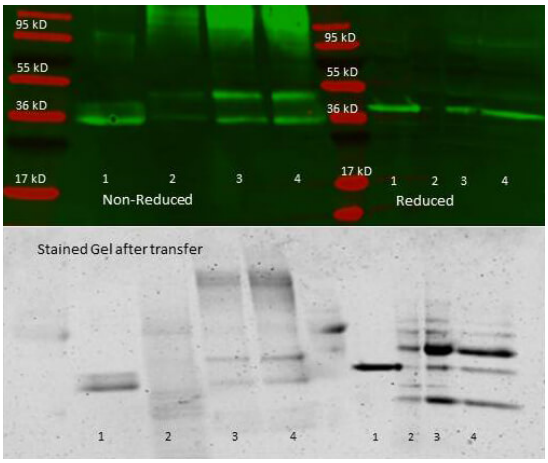
Images



Immunoprecipitation
Recognition of the RA serum reactive 40 kDa protein by antirabbit aldolase A antibody. The MG-63 cell extract (100 µl, 2 mg/ml) was immunoprecipitated with the antirabbit aldolase A antibody or with the control IgG. The immunoprecipitates were analyzed by western blotting with RA or normal serum. Upper panel: immunoprecipitation (IP) with the anti-aldolase A antibody (Ab). Lower panel: IP with the control IgG. Lane 1: antirabbit muscle aldolase A antibody. Lane 2: control IgG. Lane 3-7: RA serum. Lane 8 and 9: normal serum. The arrow shows the position of 40 kDa protein.
Fig 4. PMID: 10364915

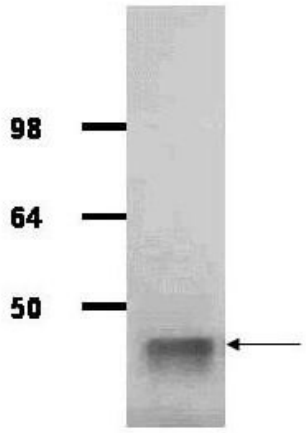


Western Blot
B), Western blot using anti-M-CK and AK antibodies demonstrating absence of M-CK and AK1 proteins in gastrocnemius homogenates from MAK=/= mice. A (weak) band migrating at slightly higher molecular weight than MM-CK represents the cross-reactive mitochondrial ScCKmit isoform. Aldolase signals are shown as controls.
Fig 1. PMID: 12730234



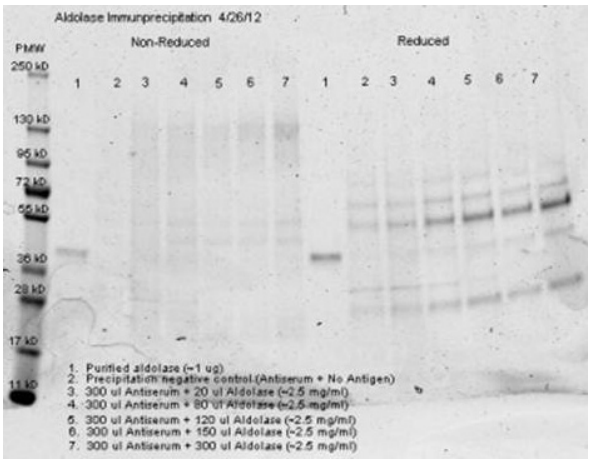
Western Blot

Anti aldolase antibody – Immunoprecipitation and Western Blot. 300 µl 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 µl antiserum with no antigen (negative control) 3. 300 µl antiserum with ~100 µl aldolase (2.5 mg/ml) 4. 300 µl antiserum with ~200 µl aldolase (2.5 mg/ml) For the precipitation, 300 ul of antiserum and an equal volume of aldolase antigen in PBS 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.



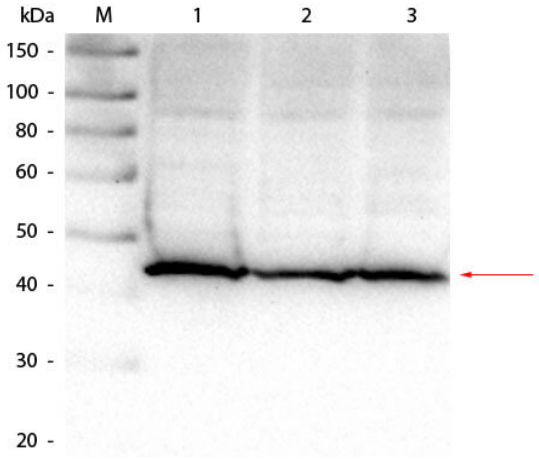
Western Blot

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.



Immunoprecipitation

Anti aldolase antibody– Immunoprecipitation- Immunoprecipitation was performed with 300 ul of anti aldolase antiserum and an equal volume of varied amounts (diluted from a stock solution of ~2.5 mg/ml) of purified 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

Western Blot of Goat anti-Aldolase Antibody. Lane 1: Hela lysate (p/n W09-000-364). Lane 2: HEK293 lysate (p/n W09 -000-365). Lane 3: Jurkat lysate (p/n W09-001-370). Load: 25 µg per lane. Primary antibody: Goat anti-Aldolase Antibody at 1:1,000 overnight at 4°C. Secondary antibody: HRP Dk-a-Gt IgG secondary antibody (p/n 605-703-002) at 1:40,000 for 30 min at RT. Block: (p/n MB-070) for 30 min at RT. Predicted/Observed size: 39 kDa, 41 kDa for Aldolase.

References

- 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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