

ab196994

Aldolase Activity Assay Kit (Colorimetric)

Instructions for Use

For the rapid, sensitive and accurate measurement of Aldolase activity in various samples.

This product is for research use only and is not intended for diagnostic use.

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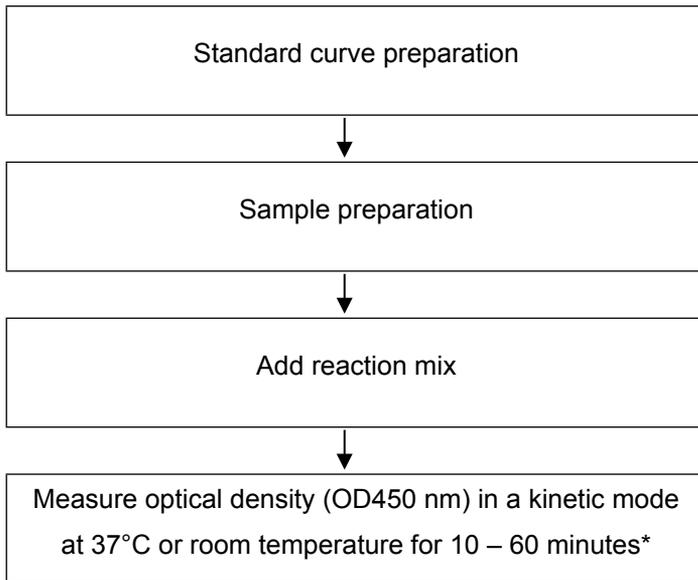
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1. OVERVIEW

Aldolase Activity Assay Kit (Colorimetric) (ab196994) is a kit where aldolase converts fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone, and through a series of reactions, reduces a nearly colorless probe to a colored product with absorbance at 450 nm. This assay kit is simple, sensitive and high-throughput adaptable. Detection limit: less than 0.1 mU of aldolase activity in a variety of samples.

Aldolase (Fructose-Bisphosphate Aldolase: EC 4.1.2.13) is an important enzyme for both glycolysis and gluconeogenesis. It catalyzes the reversible reaction of fructose-1,6-bisphosphate to glyceraldehyde-3-phosphate and dihydroxyacetone. There are 2 classes of Aldolase - class I: found in animal and plant tissues and class II: found in prokaryotes and lower eukaryotes. Class I Aldolase has 3 isozymes- Type A: found in muscle and red blood cells, Type B: found in liver and kidney and Type C: found in brain. Aldolase A deficiency leads to myopathy and hemolytic anemia. Muscle disease and liver injury can also cause increased serum aldolase. Accurate detection of aldolase activity is valuable for diagnostic and mechanistic studies.

2. ASSAY SUMMARY



**For kinetic mode detection, incubation time given in this summary is for guidance only.*

3. PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

All kit components have been formulated and quality control tested to function successfully as a kit. Modifications to the kit components or procedures may result in loss of performance.

4. STORAGE AND STABILITY

Store kit at -20°C in the dark immediately upon receipt. Kit has a storage time of 1 year from receipt, providing components have not been reconstituted.

Refer to list of materials supplied for storage conditions of individual components. Observe the storage conditions for individual prepared components in section 5.

Aliquot components in working volumes before storing at the recommended temperature. **Reconstituted components are stable for 2 months.**

5. MATERIALS SUPPLIED

Item	Amount	Storage Condition (Before Preparation)	Storage Condition (After Preparation)
Assay Buffer 4	25 mL	-20°C	-20°C / +4°C
Developer Solution III	1 vial	-20°C	-20°C
PGK Developer	1 vial	-20°C	-80°C
Aldolase Substrate	1 vial	-20°C	-20°C
NADH Standard I	1 vial	-20°C	-20°C
Aldolase Positive Control	1 vial	-20°C	-80°C

PLEASE NOTE: Assay Buffer 4 was previously labelled as Assay Buffer IV and Aldolase Assay Buffer. The composition has not changed.

6. MATERIALS REQUIRED, NOT SUPPLIED

These materials are not included in the kit, but will be required to successfully utilize this assay:

- MilliQ water or other type of double distilled water (ddH₂O)
- Cold PBS
- 100% isopropanol
- Microcentrifuge
- Pipettes and pipette tips
- Colorimetric microplate reader – equipped with filter for OD 450 nm
- 96 well plate: clear plate for colorimetric assay
- Orbital shaker
- Heat block or water bath
- Vortex
- Dounce homogenizer or pestle (if using tissue)

7. LIMITATIONS

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- Do not use kit or components if it has exceeded the expiration date on the kit labels.
- Do not mix or substitute reagents or materials from other kit lots or vendors. Kits are QC tested as a set of components and performance cannot be guaranteed if utilized separately or substituted.

8. TECHNICAL HINTS

- **This kit is sold based on number of tests. A ‘test’ simply refers to a single assay well. The number of wells that contain sample, control or standard will vary by product. Review the protocol completely to confirm this kit meets your requirements. Please contact our Technical Support staff with any questions.**
- Keep enzymes and heat labile components and samples on ice during the assay.
- Make sure all buffers and developing solutions are at room temperature before starting the experiment.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Samples generating values higher than the highest standard should be further diluted in the appropriate sample dilution buffers.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure complete removal of all solutions and buffers from tubes or plates during wash steps.
- Make sure you have the appropriate type of plate for the detection method of choice.
- Make sure the heat block/water bath and microplate reader are switched on before starting the experiment.

9. REAGENT PREPARATION

- Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 4 :

Ready to use as supplied. Equilibrate to room temperature before use. Store at 4°C or -20°C.

9.2 Aldolase Substrate:

Reconstitute with 220 μL ddH₂O. Pipette up and down to dissolve. Aliquot substrate so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. Use within two months.

9.3 PGK Developer:

Reconstitute with 220 μL Assay Buffer. Pipette up and down to dissolve. Keep on ice during the assay. Aliquot enzyme mix so that you have enough volume to perform the desired number of assays. Store aliquots at -80°C. Keep on ice while in use. Use within two months.

9.4 Developer Solution III:

Reconstitute with 220 μL ddH₂O. Pipette up and down to dissolve completely. Aliquot developer so that you have enough volume to perform the desired number of assays. Store aliquots at -20°C. Use within two months.

9.5 NADH Standard I

Reconstitute with 400 μL ddH₂O to generate 1.25 mM NADH Standard I solution. Keep on ice during the assay. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at -20°C. Keep on ice while in use. Use within two months.

9.6 Aldolase Positive Control

Reconstitute with 200 μL ddH₂O and mix thoroughly. Keep on ice during the assay. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at -80°C. Keep on ice while in use. Use within two months.

10. STANDARD PREPARATION

- Always prepare a fresh set of standards for every use.

10.1 Using 1.25mM NADH Standard I, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	Volume of Standard (μL)	Assay Buffer (μL)	Final volume standard in well (μL)	End Conc. NADH in well
1	0	125	50 μL	0 nmol/well
2	5	120	50 μL	2.5 nmol/well
3	10	115	50 μL	5 nmol/well
4	15	110	50 μL	7.5 nmol/well
5	20	105	50 μL	10 nmol/well
6	25	100	50 μL	12.5 nmol/well

Each dilution has enough amount of standard to set up duplicate reading (2 x 50 μL).

11. SAMPLE PREPARATION

General Sample information:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.
- We recommend that you use fresh samples. If you cannot perform the assay at the same time, we suggest that you complete the Sample Preparation step before storing the samples. Alternatively, if that is not possible, we suggest that you snap freeze cells or tissue in liquid nitrogen upon extraction and store the samples immediately at -80°C . When you are ready to test your samples, thaw them on ice. Be aware however that this might affect the stability of your samples and the readings can be lower than expected.

11.1 Cell (adherent or suspension) samples:

- 11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation = 1×10^6 cells).
- 11.1.2 Wash cells with cold PBS.
- 11.1.3 Resuspend cells in 100 μL ice cold Assay Buffer 4 .
- 11.1.4 Homogenize cells quickly by pipetting up and down a few times. Keep on ice for 10 minutes.
- 11.1.5 Centrifuge sample 5 minutes at 4°C at 10,000 x g using a cold microcentrifuge to remove any insoluble material.
- 11.1.6 Collect supernatant and transfer to a clean tube.
- 11.1.7 Keep on ice.

11.2 Tissue samples:

- 11.2.1 Harvest the amount of cells necessary for each assay (initial recommendation = 10 mg tissue).
- 11.2.2 Wash tissue in cold PBS.
- 11.2.3 Resuspend tissue in 100 μL ice cold Assay Buffer 4 .
- 11.2.4 Homogenize tissue with a Dounce homogenizer sitting on ice, with 10 – 15 passes. Keep on ice for 10 minutes.

11.2.5 Centrifuge sample 5 minutes at 4°C at 10,000 x *g* using a cold microcentrifuge to remove any insoluble material.

11.2.6 Collect supernatant and transfer to a clean tube.

11.2.7 Keep on ice.

11.3 Serum and Urine:

Serum and urine samples can be tested directly by adding sample to the microplate wells.

However, to find the optimal values and ensure your readings will fall within the standard values, we recommend performing several dilutions of the sample.

NOTE: We suggest using different volumes of sample to ensure readings are within the Standard Curve range.

12. ASSAY PROCEDURE

- It is recommended to assay all standards, controls and samples in duplicate.

12.1 Set up Reaction wells:

- Standard wells = 50 μ L Standard dilutions.
- Sample wells = 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer 4).
- Positive control = 2 – 10 μ L Aldolase Positive Control and adjust volume to 50 μ L/well with Assay Buffer 4).
- Background control sample wells = 1 – 50 μ L samples (adjust volume to 50 μ L/well with Assay Buffer 4).

12.2 Reaction Mix:

Prepare 50 μ L of Reaction Mix for each reaction:

Component	Reaction Mix (μ L)	Background Control Mix (μ L)
Assay Buffer 4	44	46
Aldolase Substrate	2	0
PGK Developer	2	2
Developer Solution III	2	2

Mix enough reagents for the number of assays (samples, standards and background control) to be performed. Prepare a Master Mix of the Reaction Mix to ensure consistency. We recommend the following calculation:

X μ L component x (Number samples + standards +1)

- 12.3 Add 50 μ L of Reaction Mix into each standard, sample and positive well. Mix thoroughly.
- 12.4 Add 50 μ L of Background Control Mix into each background control sample well. Mix thoroughly.

- 12.5 Measure absorbance on a microplate reader at OD=450 nm in a kinetic mode, every 2 – 3 minutes, for 10 – 60 minutes at 37°C.

NOTE: *Sample incubation time can vary depending on α -amylase activity in samples. We recommend measuring the OD in kinetic mode, and choosing two time points (T_1 and T_2) in the linear portion of the time course to calculate the aldolase activity.*

The NADH Standard Curve can be read in the Endpoint mode (i.e. at the end of incubation time).

13. CALCULATIONS

- Samples producing signals greater than that of the highest standard should be further diluted in appropriate buffer and reanalyzed, then multiplying the concentration found by the appropriate dilution factor.
- For statistical reasons, we recommend each sample should be assayed with a minimum of two replicates (duplicates).

13.1 Average the duplicate reading for each standard and sample.

13.2 Subtract the mean absorbance value of the blank (Standard #1) from all standard and sample readings. This is the corrected absorbance.

13.3 Plot the corrected absorbance values for each standard as a function of the final concentration of NADH.

13.4 Draw the best smooth curve through these points to construct the standard curve. Most plate reader software or Excel can plot these values and curve fit. Calculate the trendline equation based on your standard curve data (use the equation that provides the most accurate fit).

13.5 Extrapolate sample readings from the standard curve plotted using the following equation:

$$\text{Time point value} = \left(\frac{\text{Corrected absorbance} - (y - \text{intercept})}{\text{Slope}} \right)$$

Time point = A_1 or A_2 (and A_{BG1} or A_{BG2} for the sample background control)

13.6 Activity of aldolase is:

$$\Delta\text{OD}_{450\text{nm}} = (A_2 - A_{BG2}) - (A_1 - A_{BG1})$$

13.7 Use the $\Delta\text{OD}_{450\text{nm}}$ to obtain B pmoles of NADH:

$$\text{Aldolase Activity} = \left(\frac{B}{(T2 - T1) \times V} \right) * D$$

$$= \text{nmol/min}/\mu\text{L} = \text{mU}/\mu\text{L} = \text{U/mL}$$

Where:

B = Amount of NADH from Standard Curve (nmol)

T1 = Time of the first reading (A_1) in minutes.

T2 = Time of the second reading (A_2) in minutes.

V = sample volume added to reaction well (μL).

D = sample dilution factor.

Sample AHCY Activity can also be expressed as U/mg of tissue or protein.

Unit Definition:

1 Unit aldolase activity = amount of enzyme that generates 1.0 μmol of NADH per minute at pH 7.2 at 37°C.

14. TYPICAL DATA

TYPICAL STANDARD CURVE – Data provided for **demonstration purposes only**. A new standard curve must be generated for each assay performed.

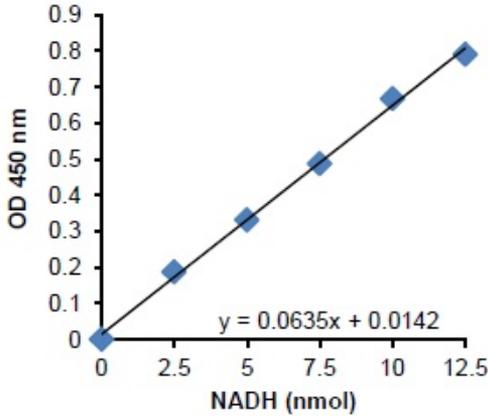


Figure 1: Typical NADH Standard Curve obtained following assay protocol.

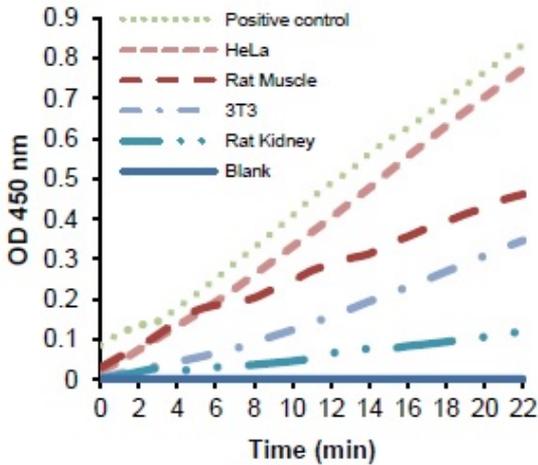


Figure 2: Aldolase Activity in rat kidney and muscle, HeLa and 3T3 cells.

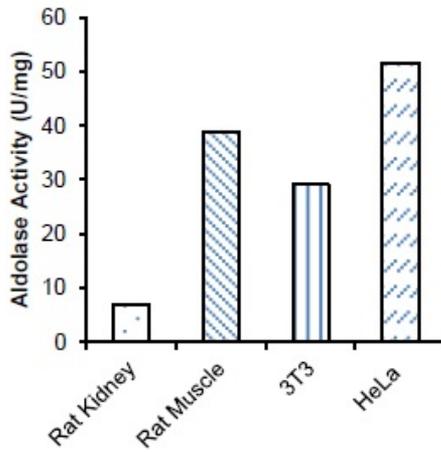


Figure 3: Relative Aldolase Activity in lysates prepared from rat kidney (14.2 μ g), rat muscle (10.64 μ g), 3T3 cells (9.97 μ g) and HeLa cells (11.35 μ g).

15. QUICK ASSAY PROCEDURE

NOTE: This procedure is provided as a quick reference for experienced users. Follow the detailed procedure when performing the assay for the first time.

- Thaw Assay buffer, solubilize rest of reagents (aliquot if necessary); get equipment ready.
- Prepare standard curve for colorimetric detection.
- Prepare samples in duplicate (find optimal dilutions to fit standard curve readings, make up to 50 μ L with Assay Buffer).
- Set up a plate for standard (50 μ L); samples (50 μ L), positive control (50 μ L) and background control (50 μ L).
- Prepare Reaction Mix (50 μ L/well) (Number samples + standards + 1).

Component	Reaction Mix (μ L)	Background Reaction Mix (μ L)
Assay Buffer 4	44	46
Aldolase Substrate	2	0
PGK Developer	2	2
Developer Solution III	2	2

- Add 50 μ L Reaction mix to standard, samples and positive control wells.
- Add 100 μ L Background Reaction mix to background controls.
- Measure output plate (A_1) at time T_1 at OD= 450 nm.
- Measure plate (A_2) at time T_2 at OD= 450 nm, while incubating plate at 37°C during 1 - 60 minutes.

16. TROUBLESHOOTING

Problem	Cause	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at room temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different 96-well plate	Colorimetric: Clear plates Fluorometric: black wells/clear bottom plate
Sample with erratic readings	Samples not deproteinized (if indicated on protocol)	Use PCA precipitation protocol for deproteinization
	Cells/tissue samples not homogenized completely	Use Dounce homogenizer, increase number of strokes
	Samples used after multiple free/ thaw cycles	Aliquot and freeze samples if needed to use multiple times
	Use of old or inappropriately stored samples	Use fresh samples or store at -80°C (after snap freeze in liquid nitrogen) till use
	Presence of interfering substance in the sample	Check protocol for interfering substances; deproteinize samples
Lower/ Higher readings in samples and Standards	Improperly thawed components	Thaw all components completely and mix gently before use
	Allowing reagents to sit for extended times on ice	Always thaw and prepare fresh reaction mix before use
	Incorrect incubation times or temperatures	Verify correct incubation times and temperatures in protocol

RESOURCES

Problem	Cause	Solution
Standard readings do not follow a linear pattern	Pipetting errors in standard or reaction mix	Avoid pipetting small volumes (< 5 μL) and prepare a master mix whenever possible
	Air bubbles formed in well	Pipette gently against the wall of the tubes
	Standard stock is at incorrect concentration	Always refer to dilutions described in the protocol
Unanticipated results	Measured at incorrect wavelength	Check equipment and filter setting
	Samples contain interfering substances	Troubleshoot if it interferes with the kit
	Sample readings above/ below the linear range	Concentrate/ Dilute sample so it is within the linear range

17. INTERFERENCES

These chemicals or biological materials will cause interferences in this assay causing compromised results or complete failure:

- RIPA buffer – contains SDS which can denature proteins and affect enzyme activity.

18. NOTES

Technical Support

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