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ab211112 Aldo-Keto Reductase (AKR) Activity Assay Kit (Colorimetric)

For the rapid, sensitive and accurate measurement of Aldo-Keto Reductase (AKR) activity in a variety of biological samples.

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

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1. Overview

Aldo-Keto Reductase (AKR) Activity Assay Kit (Colorimetric) (ab211112) provides a convenient tool for sensitive detection of Aldo-Keto Reductase (AKR) activity in animal tissue and cells, as well as biological fluids such as serum or plasma. The assay is based on the ability of AKR to reduce a general substrate and convert $NADP^+$ to NADPH, which reacts with the NADP Detection Probe to generates color that can be detected at OD 450 nm. The intensity of the color is proportional to the activity of AKR in the sample.

This assay can detect as low as 1 µU of AKR activity in samples. It has been validated with AKR1B10, AKR1C1 and AKR1C3.

Aldo-keto Reductases (AKRs) are a superfamily of NAD(P)H linked oxidoreductases which contains more than 190 members and are present in nearly all phyla. They are mainly monomeric soluble proteins (34 – 37 kD) and their common is to oxidize/reduce aldehydes or ketones to their corresponding primary or secondary alcohols. Due to their broad substrate specificity, AKRs play roles in intermediary metabolism, biosynthesis and detoxification.

In humans, AKRs are not only involved in carbonyl metabolism, but they also regulate signaling through nuclear receptors which leads to chemical carcinogenesis. Many studies demonstrate increasing expression of AKRs in cancers such as lung, liver and colon cancers.

2. Protocol Summary

Standard curve preparation

Sample preparation

Add reaction mix

Measure absorbance at OD450 nm in kinetic mode
for 10 – 120 minutes at 37°C

**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.
- We understand that, occasionally, experimental protocols might need to be modified to meet unique experimental circumstances. However, we cannot guarantee the performance of the product outside the conditions detailed in this protocol booklet.
- Reagents should be treated as possible mutagens and should be handled with care and disposed of properly. Please review the Safety Datasheet (SDS) provided with the product for information on the specific components.
- Observe good laboratory practices. Gloves, lab coat, and protective eyewear should always be worn. Never pipette by mouth. Do not eat, drink or smoke in the laboratory areas.
- All biological materials should be treated as potentially hazardous and handled as such. They should be disposed of in accordance with established safety procedures.

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 the Materials Supplied section.

Aliquot components in working volumes before storing at the recommended temperature.

Δ Note: Reconstituted components are stable for 2 months.

5. Limitations

- Assay kit intended for research use only. Not for use in diagnostic procedures.
- 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.

6. Materials Supplied

Item	Quantity	Storage temperature (before prep)	Storage temperature (after prep)
Assay Buffer 5	25 mL	-20°C	4°C / -20°C
AKR Substrate	10 mL	-20°C	4°C / -20°C
NADP Detection Probe	1 vial	-20°C	-20°C
AKR Positive Control	10 µL	-20°C	-20°C
NADPH Standard	1 vial	-20°C	-20°C

PLEASE NOTE: Assay Buffer 5 was previously labelled as Assay Buffer V and AKR Assay Buffer. The composition has not changed.

7. Materials Required, Not Supplied

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

- Multi-well spectrophotometer.
- MilliQ water or other type of double distilled water (ddH_2O)
- Cold PBS
- Anhydrous DMSO (reagent grade)
- Pipettes and pipette tips, including multi-channel pipette
- Assorted glassware for the preparation of reagents and buffer solutions
- Tubes for the preparation of reagents and buffer solutions
- 96-well clear flat bottom plate.
- Dounce homogenizer (if using tissue)

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.**
- Selected components in this kit are supplied in surplus amount to account for additional dilutions, evaporation, or instrumentation settings where higher volumes are required. They should be disposed of in accordance with established safety procedures.
- Avoid foaming or bubbles when mixing or reconstituting components.
- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps.
- Ensure all reagents and solutions are at the appropriate temperature before starting the assay.
- Samples which generate values that are greater than the most concentrated standard should be further diluted in the appropriate sample dilution buffer.
- Make sure all necessary equipment is switched on and set at the appropriate temperature.

9. Reagent Preparation

Briefly centrifuge small vials at low speed prior to opening.

9.1 Assay Buffer 5:

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

9.2 AKR Substrate:

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

9.3 NADP Detection Probe:

Reconstitute with 900 µL of ddH_2O. Pipette up and down several times to completely dissolve the pellet; do not vortex. Aliquot probe so that you have enough volume to perform the desired number of assays. Store at –20°C. Use within two months. Keep on ice while in use.

9.4 AKR Positive Control:

Ready to use as supplied. Aliquot positive control so that you have enough volume to perform the desired number of assays. Store at –20°C. Use within two months. Keep on ice while in use.

9.5 NADPH Standard:

Reconstitute NADPH Standard in 200 µL DMSO to generate 1 mM NADPH Standard solution. Aliquot standard so that you have enough volume to perform the desired number of assays. Store at –20°C. Use within 2 months. Keep on ice while in use.

10. Standard Preparation

- Always prepare a fresh set of standards for every use.
- Discard working standard dilutions after use as they do not store well.

10.1 Prepare a 0.2 mM NADPH working standard solution (1:5 dilution) by adding 65 µL 1 mM NADPH Standard to 260 µL Assay Buffer.

10.2 Using 0.2 mM NADPH working standard, prepare standard curve dilution as described in the table in a microplate or microcentrifuge tubes:

Standard #	NADPH 0.2 mM Standard (µL)	Assay Buffer (µL)	Final volume standard in well (µL)	End amount NADPH in well (nmol/well)
1	0	300	100	0
2	30	270	100	2
3	60	240	100	4
4	90	210	100	6
5	120	180	100	8

Each dilution has enough amount of standard to set up duplicate readings (2 x 100 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 your samples in liquid nitrogen upon extraction and store them 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 lysates:

11.1.1 Harvest the amount of cells necessary for each assay (initial recommendation: 1 x 10^6 cells)
11.1.2 Homogenize ~1 x 10^6 cells in 200 µL ice-cold Assay Buffer.
11.1.3 Centrifuge sample for 10 minutes at 4°C at 13,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.1.4 Collect supernatant and transfer to a new tube.
11.1.5 Keep on ice.

11.2 Tissue lysates:

11.2.1 Harvest the amount of tissue necessary for each assay (initial recommendation ~10 - 50 mg)
11.2.2 Wash tissue in cold PBS.
11.2.3 Homogenize tissue in 200 µL ice-cold Assay Buffer with a Dounce homogenizer sitting on ice, with 10 – 15 passes.
11.2.4 Centrifuge sample for 10 minutes at 4°C at 13,000 x*g* using a cold microcentrifuge to remove any insoluble material.
11.2.5 Collect supernatant and transfer to a new tube.
11.2.6 Keep on ice.

11.3 Serum samples:

Serum samples can be tested directly. No preparation is required.

Δ Note: We suggest using different volumes of sample to ensure readings are within the standard curve range.

12. Assay Procedure

- Equilibrate all materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls and samples in duplicate.
- Prepare all reagents, working standards, and samples as directed in the previous sections.

Δ Note: If you suspect your samples contain substances that can generate background, set up Sample Background Controls to correct for background noise.

12.1 Plate Loading:

- Standard wells = 100 µL standard dilutions.
- Sample wells = 2-50 µL samples, (adjust volume for all samples to 100 µL/well with Assay Buffer).

Δ Note: for serum, use 5-50 µL per well.

- Sample Background Control wells = 2-50 µL samples (adjust volume to 100 µL/well with Assay Buffer).

Δ Note: for serum, use 5-50 µL per well.

- Positive Control = 2 - 5 µL AKR Positive Control (adjust volume to 100 µL/well with Assay Buffer).

12.2 AKR Reaction Mix:

12.2.1 Prepare 100 µL of Reaction Mix and Background Control Mix for each reaction. Mix enough reagents for the number of assays to be performed. Prepare a master mix to ensure consistency.

Component	Reaction Mix (µL)	Background Control Mix (µL)
AKR Buffer	0	92
AKR Substrate	92	0
NADP Detection Probe	8	8

12.2.2 Add 100 µL of Reaction Mix into each standard, sample and positive control well.

12.2.3 Add 100 µl of Background Control Mix to Sample Background Control wells.

12.2.4 Mix well.

12.3 Measurement:

12.3.1 Measuring immediately absorbance at 450 nm in a kinetic mode for 10-120 minutes at 37°C.

Δ Note: The NADPH Standard Curve can be read in Endpoint mode (after 10 minutes of incubation).

Δ Note: Sample incubation time depends on the AKR activity in them. We recommend measuring the OD in a kinetic mode, and choosing two time points (T1 and T2) in the linear range of the standard curve to calculate the AKR activity of the samples. The NADPH Standard curve can be read in endpoint mode (ie. 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 multiply the concentration found by the appropriate dilution factor.
- Use only the linear rate for calculation.

13.1 Standard curve calculation:

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

13.1.2 Average the duplicate reading for each standard.

13.1.3 Plot standard curve readings and draw the line of the best fit to construct the standard curve (most plate reader software or Excel can do this step). Calculate the trend line equation based on your standard curve data (use the equation that provides the most accurate fit).

13.2 Measurement of Aldo-Keto Reductase activity in the sample:

13.2.1 For all reaction wells, choose two time points (T1 and T2) in the linear phase of the reaction progress curves and obtain the corresponding absorbance values at those points (OD1 and OD2).

13.2.2 Calculate ΔOD450 as follows:

$$\Delta OD450 = OD2 - OD1$$

13.2.3 If sample background control reading is significant, subtract sample background control reading from sample reading.

13.2.4 Apply the ΔOD to the NADPH Standard Curve to get B nmol of NADPH generated during the reaction time.

13.3 AKR activity (nmol/min/mL or mU/mL) in the test samples is calculated as:

$$AKR\ Activity = \left(\frac{B}{\Delta T * V}\right) * D$$

Where:

B = amount of NADPH in sample well calculated from standard curve (nmol).

ΔT = linear phase reaction time T2 – T1 (minutes).

V = original sample volume added into the reaction well (mL)

D = sample dilution factor.

Unit definition:

1 Unit of Aldo-Keto Reductase activity = amount of enzyme that generates 1.0 µmol of NADPH per min at pH8.0 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.

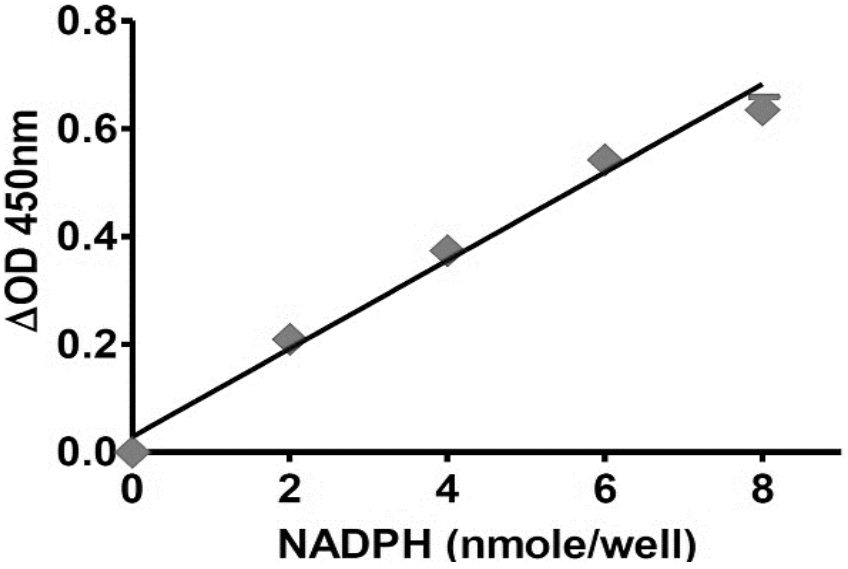

Figure 1. Typical NADPH standard curve.

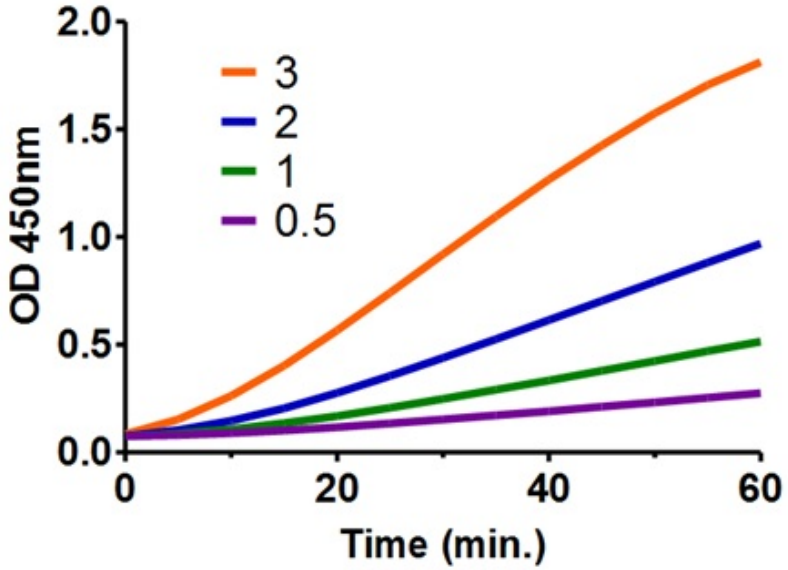

Figure 2. Kinetic curves showing AKR activity detection in increase amounts of positive control included in the kit (µL/assay).

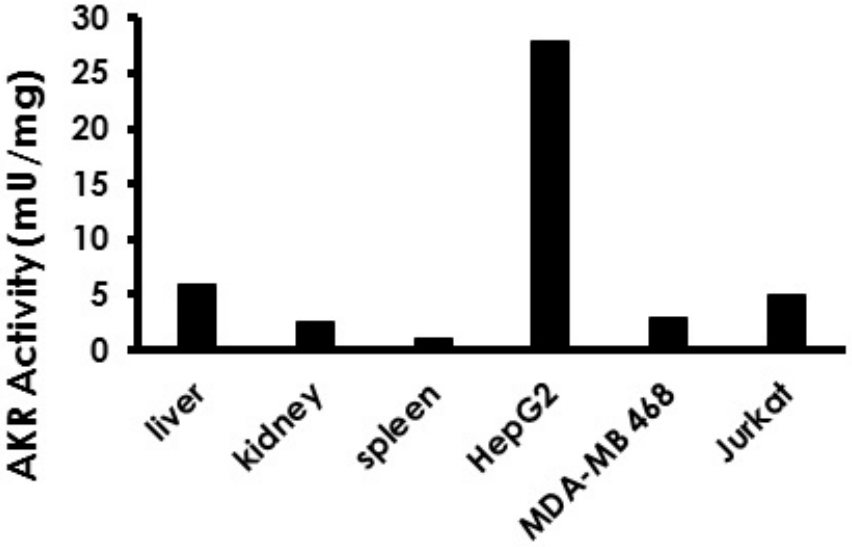

Figure 3. AKR specific activity in human tissue lysates and cell line lysates. Serial dilutions were tested to ensure the readings were within the linear range of the Standard Curve.

15. Quick Assay Procedure

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

- Prepare reagents and aliquot; get equipment ready.
- Prepare standard dilution [2 – 8 nmol/well].
- Prepare samples in optimal dilutions to fit standard curve readings.
- Set up plate in duplicate for standard (100 µL), sample background control samples (100 µL), sample (100 µL) and positive control wells (100 µL).
- Prepare a master mix for AKR Reaction Mix and Background Control Mix:

Component	Reaction Mix (µL)	Background Control Mix (µL)
AKR Buffer	0	92
AKR Substrate	92	0
NADP Detection Probe	8	8

- Add 100 µL Reaction mix to standard, sample and positive control wells.
- Add Background Control Mix to Sample Background Control wells.
- Measure absorbance immediately at OD = 450 nm in a kinetic mode for 10-120 minutes at 37 °C.

16. Troubleshooting

Problem	Reason	Solution
Assay not working	Use of ice-cold buffer	Buffers must be at assay temperature
	Plate read at incorrect wavelength	Check the wavelength and filter settings of instrument
	Use of a different microplate	Colorimetric: clear plates Fluorometric: black wells/clear bottom plates Luminometric: white wells/clear bottom plates
Sample with erratic readings	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
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

Problem	Reason	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. Notes

Technical Support

For all technical or commercial enquiries please go to:

www.abcam.com/contactus

www.abcam.cn/contactus (China)

www.abcam.co.jp/contactus (Japan)