Datasheet

amsbio

Exo enolase

Enolase activity kit

for Extracellular Vesicle characterization

For research use only, not for use in diagnostic procedures

Read the user guide prior using this product
Do not use the kit or its components beyond the indicated expiration date

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Enolase kit for Extracellular Vesicle characterization

Enolase (EC 4.2.1.11), also called 2-phospho-D-glycerate hydrolase or 2-phosphoglycerate dehydratase, is a key enzyme in glycolysis. It converts 2-phosphoglycerate to phosphoenolpyruvate (PEP) & also catalyzes the reverse reaction, PEP to 2-phosphoglycerate under anabolic conditions during gluconeogenesis. In mammals, Enolase consists of three subunits: α, β and γ that combine into 5 isozymes, 3 of which are commonly found in human tissues. This enzyme exists in all organisms, which can undergo glycolysis. Enolase activity is easily detectable in extracellular vesicles (EVs) derived from eukaryotic cells (see below) and it could be used for evaluating functionality and stability of EVs. Moreover, it's increased activity is associated with tumorigenesis and therefore precise measurement of enolase activity may be of great interest for EV-based tumor diagnosis.

AMSBIO's Enolase Activity Assay Kit measures the conversion of 2-phosphoglycerate to phosphoenolpyruvate catalyzed by the enolase enzyme. This in turn is used to generate an intermediate productwhich stoichiometrically reacts with the RedEV-probe to generate easily detectable color (OD 570 nm) orfluorescence (Ex/Em = 535/587 nm) signal. The detection limit of the kit is approximately 0.04 mU.

Applications

- Evaluation of enolase activity in various cell-derived EVs
- Evaluation of the functionality and stability of various cell-derived EVs
- Mechanistic studies of EVs of cancer origin
- Suitable for measuring enolase activity from fresh, frozen or lyophilized cell-derived EVs

Compatibility

- Suitable for measuring enolase activity in fresh, frozen or lyophilized EVs.
- Compatible with EVs isolated by ultracentrifugation, size exclusion chromatography, ultrafiltration, precipitation reagents (PEG based).

Product content

Components	Quantity
Assay Buffer	25 ml
RedEV Probe	0.2 ml
Substrate Mix (Lyophilized)	1 vial
Converter (Lyophilized)	1 vial
Developer (Lyophilized)	1 vial
H_2O_2 standard (0.88 M)	0.1 ml
Assay Positive Control (Lyophilized)	1 vial
EV Positive Control (Lyophilized)	2 vials

Reagents and equipment not supplied

- 96-well clear or black flat-bottom plates.
- Spectrophotometer for 96-well plates.

Storage and handling

- Shippment: kit is shipped at controled temperature (4 - 8° C) with ice packs.
- Storage: refer to the guide below.

Components	Procedure
Assay Buffer	Store at -20° C
RedEV Probe	Store at -20° C
Substrate Mix (Lyophilized)	Briefly centrifuge the vial before opening. Reconstitute in 220 µl of Assay Buffer and keep at -20° C. Use within two months after reconstitution
Converter (Lyophilized)	Briefly centrifuge the vial before opening. Reconstitute in 220 µl of Assay Buffer and keep at -20° C. Use within two months after reconstitution
Developer (Lyophilized)	Briefly centrifuge the vial before opening. Reconstitute in 220 µl of Assay Buffer and keep at -20° C. Use within two months after reconstitution
H_2O_2 standard (0.88 M)	Store at -20° C
Assay Positive Control (Lyophilized)	Briefly centrifuge the vial before opening. Reconstitute in 100 µl of Assay Buffer and keep at -20° C. Use within two months after reconstitution
EV Positive Control (Lyophilized)	Briefly centrifuge the vial before opening. Reconstitute in 100 µl of MilliQ water pipetting up and down 10- 15 times. Vortex for 60 seconds and briefly spin the tube to collect the solution on the bottom of the vial. Divide in 10 aliquots of 10 µl and store at -20° C

Activity assay protocol

1. Add $1x10^8$ to 1×10^{10} particles per well and adjust final volume to 50 µl withAssay Buffer.

Note: Enolase activity varies depending on the source of EVs (see figure below). We suggest determining the optimal concentration for each EV source so that it would be in the range of standard curve.

2. H_2O_2 Standard Curve: Dilute H_2O_2 standard to 10 mM by adding 4 µl of 0.88 M H_2O_2 Standard into 348 µl of deionized water (MilliQ). Dilute 10 mM H_2O_2 standard further to 1 mM by adding 100 µl of 10 mM H_2O_2 into 900 µl of MilliQ water.

- For colorimetric assay, add 0, 2, 4, 6, 8 and 10 µl of 1 mM H_2O_2 standard into a series of wells in a 96-well plate to generate 0, 2, 4, 6, 8 and 10 nmol/well of H_2O_2 standard. Adjust the volume to 50 µl/well with MilliQ water.
- For fluorometric assay, dilute the 1 mM H_2O_2 to 0.1 mM by adding 100 µl of 1 mM H_2O_2 into 900 µl MilliQ water. Add 0, 2, 4, 6, 8 and 10 µl of 0.1 mM H_2O_2 standard into a series of wells in a 96-well plate to generate 0, 200, 400, 600, 800 and 1000 pmol/well of H_2O_2 Standard. Adjust the volume to 50 µl/well with MilliQ water.
- Note: Prepare working solution of H_2O_2 standard immediately before use.

3. Assay positive control: Dilute Assay Positive Control 100 times by adding 10 µl of Positive Control into 990 µl Assay Buffer.

- For colorimetric assay: add 1-10 µl of diluted Assay Positive Control into desired well(s) and adjust the final volume to 50 µl with Assay Buffer.
- For fluorometric assay: Dilute the 100 times diluted Assay Positive Control further 10 times by adding 50 µl of 100 times diluted Assay Positive Control into 450 µl Assay Buffer. Add 1-10 µl of 1000 times diluted Assay Positive Control into desired well(s) and adjust the final volume to 50 µl with Enolase Assay Buffer.

4. EV Positive Control: Add 5 - 10 µl of EV Positive Control into desired well(s) and adjust the final volume to 50 µl with Enolase Assay Buffer.
5. Reaction Mix: For each well, prepare 50 µl reaction mix as follows:

Component	**Amount in µl**
Assay Buffer	42
Substrate Mix	2
Converter	2
Developer	2
RedEV Probe	2
Note: for fluorimetric assay, dilute the RedEV Probe 10x with Assay Buffer	

6. Measurement: Measure the absorbance (OD 570 nm) or fluorescence (Ex/Em 535/587 nm) in kinetic mode for 20 – 90 minutes at 25° C.

Note: Incubation time depends on the Enolase activity in the samples and therefore kinetic mode is recommended. Choose at least two time points in the linear range to calculate the Enolase activity in the samples. H_2O_2 standard curve can be read at the beginning or at the end of the kinetic measurement – i.e. endpoint mode.

7. Calculation: Subtract 0 Standard reading from all readings. Plot the H_2O_2 standard curve to get to get B nmol or B pmol of H_2O_2 generated by Enolase during the reaction time (ΔT = T2 - T1).

Sample Enolase Activity = B/(ΔT x V) x D = nmol/min/l or pmol/min/µl = mU/µl or µU/µl = U/ml or mU/ml

Where:

B = H_2O_2 amount from standard curve (nmol or pmol)

ΔT = Reaction time (min.)

V = Sample volume added into the reaction well (µl)

D = Dilution Factor

Unit Definition: One milliunit of Enolase is the amount of enzyme that will generate 1.0 nmol of H_2O_2 per min. at pH 7.2 at 25°C.

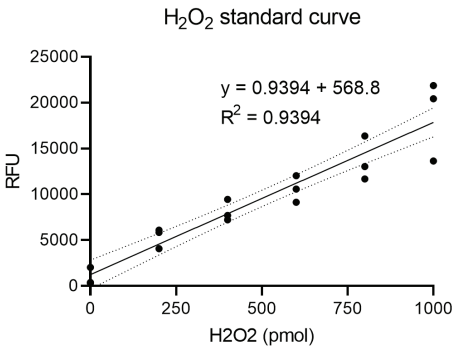

FIGURE 1: H_2O_2 standard curve for fluorometric enolase activity assay. Symbols are biological repeats, solid line shows simple linear regression analyses and dashed lines are 95% confidence intervals.

Enolase activity in EVs purified from cancer cell lines

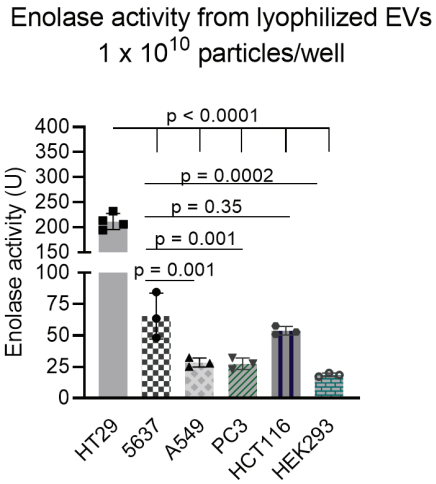

FIGURE 2: Enolase activity in EVs isolated from conditioned media of various cell lines. $1x10^{10}$ particles were used and the enolase activity was calculated based on the standard curve. Statistical analyses with one-way ANOVA and Dunnett's multiple comparison test. Symbols are biological repeats, bars indicate means and error-bars are SDs.

Enolase activity over time

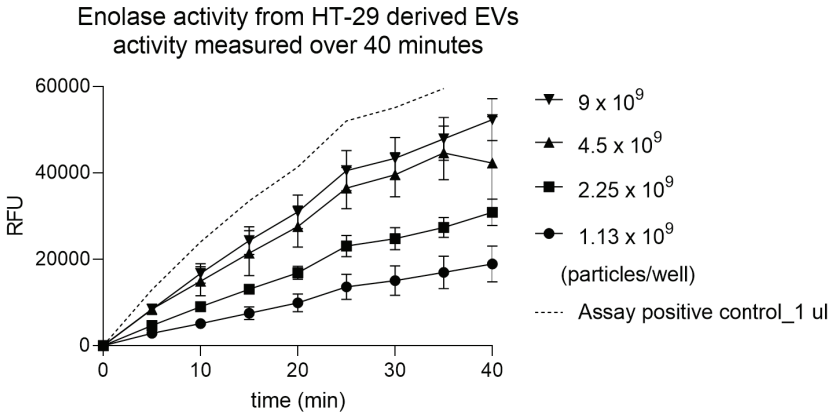

FIGURE 3: Enolase activity from HT-29 derived EVs measured over time.
Assay positive control (dashed line) up to 35 minutes is added for reference

Enolase activity and stability of EV positive control

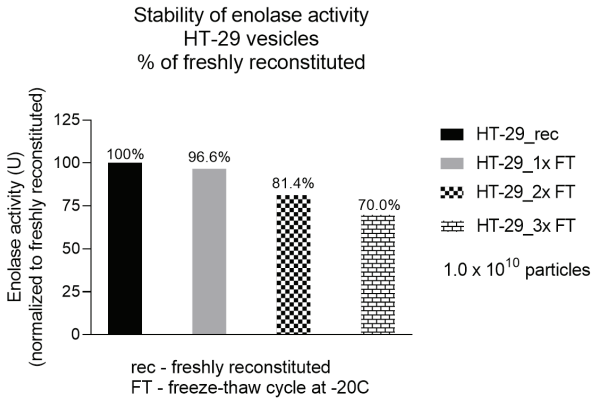

FIGURE 4: Stability of enolase activity in extracellular vesicles (EVs). Lyophilized HT-29 EVs were reconstituted in MilliQ water and 1, 2 or 3 freeze-thaw cycles at -20° C were performed. Enolase activity was normalized relative to the freshly reconstituted EVs (rec). One freeze-thaw cycle does not affect the enolase activity compared to the freshly reconstituted sample.

Related products

Code	Description
K1201 or K1202	ExoPure for Extracellular Vesicle immunocapture and quantifica tion from human plasma and urine. EV detection performed with anti-human CD9 antibody and anti-mouse HRP conjugated.
K1203	ExoPure for Extracellular Vesicle immunocapture and quantification from human serum. EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP
K1205	ExoPure for Extracellular Vesicle immunocapture and quantification from cell culture media. EV detection performed with anti-human CD9 biotin-conjugated antibody and Streptavidin-HRP
Please Enquire	Custom made ExoPure for Specific Extracellular Vesicle immunocapture and quantification.

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