ab239728 Total Polyamine Assay Kit (Fluorometric)

For rapid, sensitive and accurate measurement of polyamine content of various tissues or cells extracts.
For research use only - not intended for diagnostic use.

Background: Total Polyamine Assay Kit (ab239728) enables the rapid determination of polyamine concentration in biological samples. A selective enzyme mix acts on polyamines, generating hydrogen peroxide that is then reacted with a fluorometric probe (ex/Em = 535-587 nm) to yield a signal proportional to the amount of polyamine present. The kit includes a proprietary Sample Clean-Up reagent for pre-treating samples to eliminate common metabolites found in biological samples that may interfere with the assay or increase sample background. The assay is rapid, simple, and high throughput compatible, and can detect polyamine concentrations as low as 0.1 µM in tissue lysates and other samples, such as saliva.

Assay Summary:

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 samples, sample background, and standard curve.

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Prepare reaction mix and add 50 µL to each well.

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Incubate plate 60-90 mins at 37°C, protected from light.

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Measure fluorescence (Ex/Em = 535/587 nm).

QUICK ASSAY PROCEDURE

- Thaw and/or reconstitute reagents where applicable and prepare equipment.
- Prepare samples in duplicate.
- Prepare standard curve and make dilution to OxiRed™ Probe.
- Set up plate for standards, samples, and background controls.
- Prepare Reaction Mix and Background Control Mix.
- Add 50 µL Reaction Mix to each Standard, Sample, and Blank wells.
- Add 50 µL of Background Control Mix to Background and Reagent Control wells.
- Incubate plate at 37°C for 60-90 minutes, then measure fluorescence (Ex/Em 535/587 nm) in endpoint mode.
- Alternatively, immediately measure fluorescence (Ex/Em=535/587 nm) in kinetic mode for 60-90 minutes at 37°C directly after adding Reaction Mixes.

Precautions & Limitations:

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.
- 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.

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. Reconstituted components are stable for 2 months. Do not use kit or components if they have exceeded the expiry date.

Materials Supplied:

Item	Quantity	Storage Temperature (on receipt)	Storage temperature (reconstituted)
Polyamine Assay Buffer	25 mL	-20°C	+4°C to -20°C
OxiRed™ Probe	200 µL	-20°C	-20°C
Converter Mix K	1 vial	-20°C	-20°C
Developer Solution V	1 vial	-20°C	-20°C
Sample Clean-Up Mix I	1 vial	-20°C	-20°C
Polyamine Standard	1 vial	-20°C	-20°C

Note: Converter Mix K was previously labelled as Converter Enzyme XIII or Polyamine Enzyme Mix. OxiRed™ Probe was previously labelled as Polyamine Probe (in DMSO). The component names have changed, but the kit mechanism of detection remains unchanged.

Materials Required, Not Supplied:

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

- Microplate reader capable of measuring fluorescence at Ex/Em = 535/587 nm
- 96 well black plate with flat bottom (colorimetric assay
- Microcentrifuge
- Dounce homogenizer (tissue samples)
- 10 kDa spin column.
- MilliQ water or other type of double distilled/deionized water (ddH2O)
- Anhydrous DMSO

Reagent Preparation:

- Briefly centrifuge small vials at low speed prior to opening.
- Equilibrate reagents to room temperature before use.
- Aliquot reagents so that you have enough volume to perform the desired number of assays.

Polyamine Assay Buffer: Allow to warm to room temperature (RT) before use. Store at +4°C to -20°C.

OxiRed™ Probe: Provided as a solution in DMSO. Divide into aliquots and store at -20°C, protected from light. Prior to use, warm solution to room temperature and ensure all stock is thawed. After use, promptly retighten cap to minimize absorption of airborne moisture.

Converter Mix K: Reconstitute with 220 µL Polyamine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Developer Solution V: Reconstitute with 220 µL Polyamine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Sample Clean-Up Mix I: Reconstitute with 220 µL Polyamine Assay Buffer. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Polyamine Standard: Reconstitute with 1000 µL of ddH_2O and mix thoroughly to generate a 100 mM Polyamine Standard solution. Aliquot and store at -20°C. Keep on ice while in use and use the reconstituted stock within two months.

Standard Preparation:

- Always prepare a fresh set of standards for every use.
- Diluted standard solution is unstable and must be used within 4 hours.
- Each dilution has enough standard to set up duplicate readings (2 x 50 µL).
- If your sample readings fall out the range of your fluorometric standard curve, you might need to adjust the dilutions and create a new standard curve.

Prepare Polyamine Standard as follows:

1. Dilute the 100 mM Polyamine Standard by combining 10 µL with 990 µL ddH_2O to generate a 1 mM solution.
2. Further dilute the 1 mM solution by adding 50 µL to 950 µL ddH_2O, generating a 50 µM Polyamine Standard working solution.
3. Add 0, 2, 4, 6, and 10 µL of the 50 µM Polyamine Standard solution into a series of wells in a black 96-well plate to generate 0, 100, 200, 300, 400, and 500 pmol/well of Polyamine Standard. Bring the total volume of each well to 50 µL with Polyamine Assay Buffer.

Standard #	Volume of 50 µM Standard (µL)	Assay Buffer (µL)	Polyamine Standard/well (pmol)
1	10	40	500
2	8	42	400
3	6	44	300
4	4	46	200
5	2	48	100
6	0	50	0

Sample Preparation:

- We recommend performing several dilutions of your sample to ensure the readings are within the standard value range.

1. 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, 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.

Tissue Samples and Cultured Cells:

1. Add 50 µL of ice-cold Polyamine Assay Buffer per 10 mg of sample (wet weight) or ~ $1X10^6$ pelleted cells.
2. Homogenize samples on ice using a Dounce homogenizer.
3. Centrifuge at 10,000 x *g* for 5 minutes at 4°C. Collect the supernatant.
4. Add 2 µL of Sample Clean-Up Mix I per 100 µL lysate (or saliva) and incubate for 30 minutes at RT.
5. Transfer sample to a 10 kDa MWCO filter and filter by centrifugation at 10,000 x *g* for 10 minutes. Collect the resulting filtrate and add 2-20 µL to desired wells of a black 96-well plate.
6. Adjust the volume to 50 µL per well with Polyamine Assay Buffer.
7. For each sample, prepare identical background control reactions in separate wells.

Note: Once treated with Sample Clean-Up Mix I and filtered, cell and tissue lysates can be stored at -80°C.

Assay Procedure:

- Keep enzymes and heat labile components and samples on ice during the assay.
- Equilibrate all other materials and prepared reagents to room temperature prior to use.
- We recommend that you assay all standards, controls, and samples in duplicate.

1. Dilute OxiRed Probe 10-fold with anhydrous DMSO (i.e. mix 5 µL OxiRed™ Probe with 45 µL DMSO) immediately prior to use. Prepare enough for the number of assays to be performed, including Polyamine Standard curve wells.
2. Prepare 50 µL Reaction Mix for each well to be assayed as per the table below and mix well. Add 50 µL of Reaction Mix into Standard and Sample wells.
3. For Sample Background wells, mix and add 50 µL of the Sample Background Mix to each well.

Component	Reaction Mix (µL)	Background Mix (µL)
Polyamine Assay Buffer	44	46
Converter Mix K	2	0
Developer Solution V	2	2
Diluted OxiRed Probe	2	2

4. Incubate at 37°C for 60-90 minutes protected from light. After incubation, measure fluorescence (Ex/Em = 535/587 nm) in endpoint mode.
5. Alternatively, immediately read plate after Reaction Mixes have been added at fluorescence (Ex/Em = 535/587 nm) at 37°C in kinetic mode for 60-90 minutes.

Calculations:

- For samples producing signals greater than that of the highest standard: dilute further in appropriate buffer and reanalyze. Multiply the concentration found by the appropriate dilution factor.

1. Subtract 0 pmole Polyamine Standard reading from all standard curve readings and plot the background-subtracted Polyamine Standard curve.
2. 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).
3. Calculate the corrected fluorescence of the test samples ΔRFU = RFU_{sample} – $RFU_{background}$.
4. Apply the corrected ΔRFU value to the Polyamine Standard Curve to get *B* pmole polyamines in the well:

$$Sample\ Polyamine\ Concentration = \left(\frac{B}{V}\right)\ x\ D = \text{pmol/µL} = \text{µM}$$

Where:
B = amount of polyamine in the sample well from standard curve (pmole)
V = sample volume added to the reaction well (µL)
D = sample dilution factor (if applicable)

Technical Hints

For additional helpful hints and tips on using our assay kits please visit:
https://www.abcam.com/en-us/support/product-support

Technical Support

For technical or commercial enquiries please go to:
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