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ab239728
Total Polyamine Assay Kit

For the measurement of polyamine content of various tissues/cell extracts.

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

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1. Overview

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 in order 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.

2. Protocol Summary

Prepare samples, sample background and standard curve.

Prepare reaction mix by diluting Polyamine Porbe 10-fold with anhydrous DMSO.

Add 50 µL of the Reaction Mix to each well.

Incubate the plate for 30 min at 37 37°C, protected from light.

Read the fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells.

3. General guidelines, precautions, and troubleshooting

1. Please observe safe laboratory practice and consult the safety datasheet.
2. For general guidelines, precautions, limitations on the use of our assay kits and general assay troubleshooting tips, particularly for first time users, please consult our guide: www.abcam.com/assaykitguidelines
3. For typical data produced using the assay, please see the assay kit datasheet on our website.

4. Materials Supplied, and Storage and Stability

4. Store kit at -20°C in the dark immediately upon receipt and check below in Section 6 for storage for individual components. Kit can be stored for 1 year from receipt, if components have not been reconstituted.
5. Aliquot components in working volumes before storing at the recommended temperature.

Item	Quantity	Storage condition
Assay Buffer 42	25 mL	-20°C
OxiRed™ Probe	0.2 mL	-20°C
Converter Mix K	1 vial	-20°C
Developer Solution V	1 vial	-20°C
Sample Clean-Up Mix I	1 vial	-20°C
Polyamine Standard	1 vial	-20°C

PLEASE NOTE: Assay Buffer 42 was previously labelled as Assay Buffer XLII and Polyamine Assay Buffer, and Converter Mix K as Converter Enzyme XIII and Polyamine Enzyme Mix. OxiRed™ Probe was previously labelled as OxiRed Probe and Polyamine Probe (in DMSO). The composition has not changed.

5. Materials Required, Not Supplied

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

6. 96-well plate with flat bottom.
7. Multi-well spectrophotometer.
8. 10 kDa Spin Column.

6. Reagent Preparation

10. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
11. Prepare only as much reagent as is needed on the day of the experiment.

6.1 Assay Buffer 42:

Allow to warm to room temperature (RT) before use. Store at 4°C, protected from light.

6.2 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. After use, promptly retighten cap to minimize adsorption of airborne moisture.

6.3 Converter Mix K:

Reconstitute each vial with 220 µl Assay Buffer 42. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.4 Developer Solution V:

Reconstitute each vial with 220 µl Assay Buffer 42. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.5 Sample Clean-Up Mix I:

Reconstitute each vial with 220 µl Assay Buffer 42. Aliquot and store at -20°C. Keep on ice while in use and use reconstituted aliquots within two months.

6.6 Polyamine Standard:

Reconstitute with 100 µL of ddH_2O and mix thoroughly to generate a 100 mM Polyamine Standard solution. Aliquot and store at -20°C. Use within two months.

7. Standard Preparation

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

7.1 Dilute the 100 mM Polyamine Standard by combining 10 µl with 990 µl dH_2O to generate a 1 mM solution.

7.2 Further dilute the 1 mM solution by adding 50 µl to 950 µl dH_2O, yielding a 50 µM Polyamine Standard working solution.

7.3 Add 0, 2, 4, 6, 8, and 10 µl of 50 µM working solution into a series of wells in a black 96-well plate to generate 0, 100, 200, 300, 400 and 500 pmol per well of Polyamine Standard. Bring the total volume of each well to 50 µl with Assay Buffer 42.

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

8. Sample Preparation

8.1 For tissues and cultured cells: add 50 µl of ice-cold Assay Buffer 42 per 10 mg of sample (wet weight) or ~1 x 10^6 pelleted cells.

8.2 Homogenize samples on ice using a Dounce homogenizer.

8.3 Centrifuge at 10,000 x *g* for 5 min at 4°C. Collect the supernatant.

8.4 Add 2 µl Sample Clean-Up Mix I per 100 µl lysate (or saliva) and incubate for 30 min at RT.

8.5 Transfer sample to a 10 kD MWCO filter and filter by centrifugation at 10,000 x *g* for 10 min. Collect the resultant filtrate and add 2-20 µl to desired wells of a black 96-well plate

8.6 Adjust the volume to 50 µl per well with Assay Buffer 42.

8.7 For each sample, prepare identical background control reactions in separate wells.

ΔNotes:

- Once treated with Sampe Clean-Up Mix I and filtered, cell and tissue lysates can be stored at -80°C for future experiments.
- For unknown samples, we recommend doing a pilot experiment testing several doses to ensure that readings are within the range of the standard curve.

9. Assay Procedure

9.1 Dilute OxiRed™ Probe 10-fold with anhydrous DMSO (i.e. mix 5 µl OxiRed™ Probe with 45 µl DMSO) immediately prior to use. Mix enough reagents for the number of assays to be performed, including Polyamine Standard curve wells.

9.2 Prepare 50 µL Reaction Mix for each well to be assayed as per the table and mix well. Add 50 µL of Reaction Mix into Standard, and sample wells. Mix well.

9.3 For Sample Background wells, mix and add 50 µl of the Sample Background Mix to each well.

	Reaction Mix	Background Control Mix
Assay Buffer 42	44 µL	46 µL
Converter Mix K	2 µL	-
Developer Solution V Mix	2 µL	2 µL
Diluted OxiRed™ Probe	2 µL	2. µL

9.4 Incubate the plate for 30 min at 37°C, protected from light and read the fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells in endpoint mode.

10. Data Analysis

10.1 Subtract 0 pmole Polyamine Standard reading from all Standard curve readings. Plot the background-subtracted Polyamine Standard Curve and calculate the slope.

10.2 If sample background control reading is significant, subtract the background control reading from its paired sample reading.

10.3 Calculate the corrected fluorescence of the test samples $\Delta RFU = RFU_{sample} - RFU_{background}$.

10.4 Apply the corrected ΔRFU value to the Polyamine Standard Curve to get *B* pmole polyamines in the well.

Sample Polyamine Concentration (C) = (B/V) X D = pmol/µL = µM

Where: **B** is amount of Polyamine in the sample well from Standard Curve (pmol)

V is sample volume added into the reaction well (µL)

D is sample dilution factor (if applicable)

11. Typical Data

Typical data provided **for demonstration purposes only**.

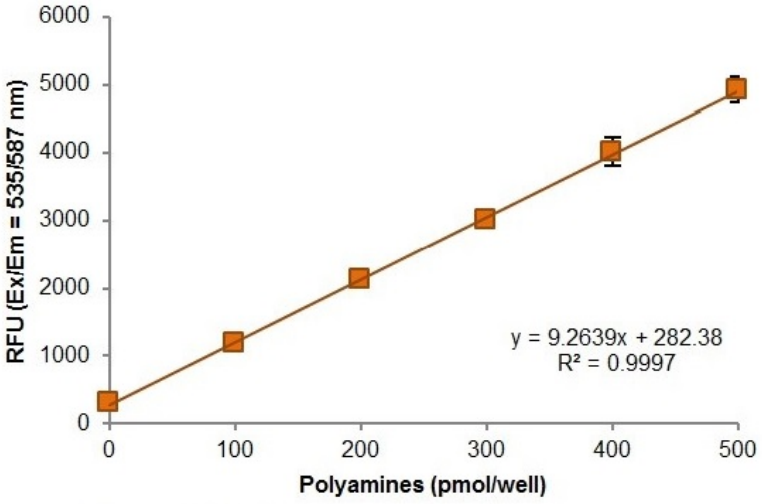

Figure 1. Polyamine Standard Curve.

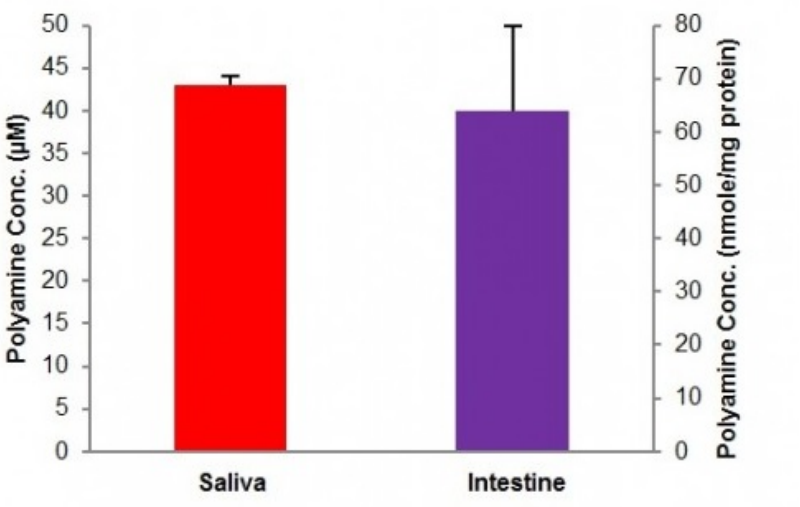

Figure 2. Determination of total polyamine concentration in saliva (determined to be 43.0 ± 8.9 µM) and intestinal tissue lysate (63.9 ± 16.0 nmole/mg protein). For this experiment, 100 mg rat intestine was homogenized and prepared according to the kit protocol. Saliva (2 µl) and intestinal lysate were treated with Sample Clean-Up Mix I. Values are mean ± standard deviation of at least three independent determinations

12. 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)