3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Total Polyamine Assay Kit

Catalog Number **MAK349**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

Polyamines are small organic molecules bearing two or more primary amine moieties. Biogenic polyamines such as spermidine, putrescine, and spermine function as transcellular signaling molecules and are involved in diverse biological processes. Although small amounts of polyamines are synthesized in cells, larger quantities are often encountered as a result of putrefaction and decay, as they are the direct product of decarboxylation of amino acids such as methionine, lysine, and arginine. The enzyme ornithine decarboxylase generates the polyamine putrescine, which is not only responsible for a repulsive odor but is also implicated in cancer.

Intracellular polyamines readily bind DNA and are critical to preventing oxidative DNA damage and directing DNA double-strand break repair pathways. Polyamine levels decline with age and dietary supplementation of the polyamine spermidine has recently been shown to reduce age-related oxidative stress and extend lifespan in mouse models of aging.

The Total Polyamine Assay Kit enables the rapid determination of polyamine concentration in biological samples. A selective enzyme mix acts on polyamines, generating hydrogen peroxide, which reacts with a fluorometric probe (λ_{ex} = 535 nm/λ_{em} = 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.

Components

The kit is sufficient for 100 fluorometric assays in 96 well plates.

Polyamine Assay Buffer Catalog Number MAK349A	25 mL
Polyamine Probe (in DMSO) Catalog Number MAK349B	200 μL
Polyamine Enzyme Mix Catalog Number MAK349C	1 vial
Polyamine Developer Catalog Number MAK349D	1 vial
Sample Clean-Up Mix Catalog Number MAK349E	1 vial
Polyamine Standard Catalog Number MAK349F	1 vial

Reagents and Equipment Required but Not Provided.

1. Pipetting devices and accessories (e.g., multichannel pipettor)
2. Black flatbottom 96 well plates
3. Fluorescence multiwell plate reader, capable of 37 °C temperature setting
4. Refrigerated microcentrifuge capable of RCF ≥10,000 × *g*
5. Dounce tissue grinder set (Catalog Number D9063 or equivalent)
6. Corning® Spin-X® UF concentrators (Catalog Number CLS431478)
7. Anhydrous Dimethylsulfoxide (DMSO) (Catalog Number 276855)

Precautions and Disclaimer

For R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability
The kit is shipped on wet ice. Store components at –20 °C, protected from light upon receiving. Briefly centrifuge small vials prior to opening.

Preparation Instructions.
Polyamine Assay Buffer – Allow to warm to room temperature prior to use.

Polyamine 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.

Polyamine Enzyme Mix, Polyamine Developer, and Sample Clean-Up Mix – Reconstitute each vial with 220 µL of Polyamine Assay Buffer. Aliquot and store at –20 °C. Keep on ice while in use and use reconstituted aliquots within two months.

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

Procedure
Sample Preparation
Note: For unknown samples, it is suggested to perform a pilot experiment testing several doses to ensure that readings are within the range of the standard curve.
1. For tissues and cultured cells, add 50 µL of ice-cold Polyamine Assay Buffer per 10 mg of sample (wet weight) or ~1×10^6 pelleted cells.
2. Homogenize samples on ice using a Dounce homogenizer.
3. Centrifuge at 10,000 × *g* for 5 minutes at 4 °C.
4. Collect the supernatant.
5. Add 2 µL of Sample Clean-Up Mix per 100 µL of lysate (or saliva) and incubate for 30 minutes at room temperature.
6. Transfer sample to a Corning Spin-X UF concentrators and filter by centrifugation at 10,000 × *g* for 10 minutes.
   Note: Once treated with Sample Clean-up Mix and filtered, cell and tissue lysates can be stored at –80 °C for future experiments.
7. Collect the resultant filtrate and add 2–20 µL to desired wells of a black 96 well plate.
8. Adjust the volume to 50 µL per well with Polyamine Assay Buffer.
9. For each sample, prepare identical background control reactions in separate wells.

Standard Curve Preparation
1. Dilute the 100 mM Polyamine Standard by combining 10 µL with 990 µL of ultrapure water to generate a 1 mM solution.
2. Further dilute the 1 mM solution by adding 50 µL to 950 µL of ultrapure water, yielding a 50 µM Polyamine Standard working solution.
3. Prepare Polyamine Standards according to Table 1. Mix well.

Table 1.
Preparation of Polyamine Standards

Well	50 µM Premix	Polyamine Assay Buffer	Polyamine (pmole/well)
1	0 µL	50 µL	0
2	2 µL	48 µL	100
3	4 µL	46 µL	200
4	6 µL	44 µL	300
5	8 µL	42 µL	400
6	10 µL	40 µL	500

Reaction Mixes
Dilute Polyamine Probe 10-fold with anhydrous DMSO (i.e., mix 5 µL of Polyamine Probe with 45 µL of DMSO) immediately prior to use. Mix enough reagents for the number of assays to be performed, including Polyamine Standard curve wells. For each test sample well, prepare 50 µL of Reaction Mix according to Table 2.

Table 2.
Preparation of Reaction Mixes

Reagent	Reaction/ Standard Mix	Sample Background Mix
Polyamine Assay Buffer	44 µL	46 µL
Polyamine Enzyme Mix	2 µL	–
Polyamine Developer Mix	2 µL	2 µL
Diluted Polyamine Probe	2 µL	2 µL

Mix and add 50 µL of the Reaction Mix to each well containing standards and test samples. For Sample Background wells, mix and add 50 µL of the Sample Background Mix to each well.

Measurement
Incubate the plate for 30 minutes at 37 °C, protected from light and read the fluorescence at λ_{ex} = 535 nm /λ_{em} = 587 nm for all reaction, sample background, and standard curve wells in endpoint mode.

Results

1. Subtract the 0 pmole Polyamine Standard reading from all standard curve readings. Plot the background-subtracted Polyamine Standard Curve and calculate the slope.
2. If sample background control reading is significant, subtract the background control reading from its paired sample reading.
3. Calculate the corrected fluorescence of the test samples $\Delta RFU = RFU_{sample} - RFU_{background}$.
4. Apply the corrected ΔRFU value to the Polyamine Standard Curve to get B pmole polyamines in the well.

Sample Polyamine Concentration (pmole/μL or μM) =

$$(B/V) \times D$$

where:
B = Polyamine amount from Standard Curve (in pmole)
V = sample volume added into the reaction well (in μL)
D = sample dilution factor (if applicable)

Figure 1.
Typical Polyamine Standard Curve

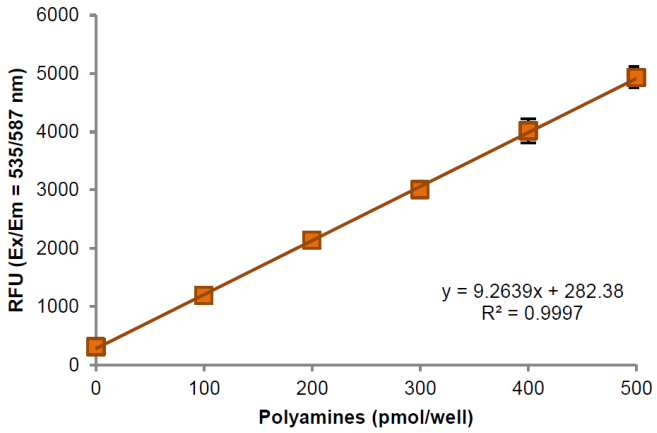
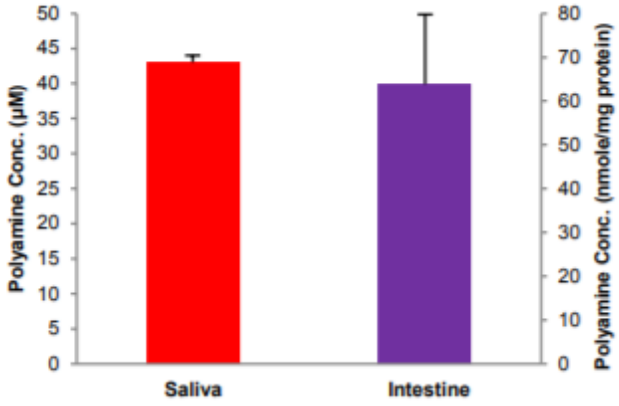


Figure 2.
Total Polyamine Concentration in Saliva and Intestinal Tissue Lysate



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 procedure. Saliva (2 μL) and intestinal lysate were treated with Sample Clean-Up Mix. Values are mean ± standard deviation of at least three independent determinations.

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