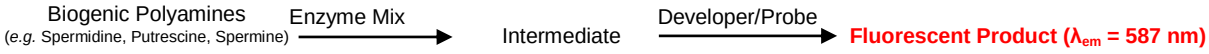
Gentaur Europe BVBA Voortstraat 49, 1910 Kampenhout BELGIUM
Tel 0032 16 58 90 45 info@gentaur.com

Total Polyamine Assay Kit (Fluorometric)

(Catalog # K475-100; 100 assays; Store at -20°C)

I. Introduction:

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 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. BioVision's Total Polyamine Detection Kit 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.



II. Applications:

- Measurement of polyamine content of various tissues/cell extracts
- Determination of polyamine concentration in biological fluids

III. Sample Type:

- Animal tissues (*e.g.* intestine, lung)
- Biological fluids (*e.g.* saliva)

IV. Kit Contents:

Components	K475-100	Cap Code	Part Number
Polyamine Assay Buffer	25 ml	WM	K475-100-1
Polyamine Probe (in DMSO)	200 µl	Red	K475-100-2
Polyamine Enzyme Mix	1 vial	Purple	K475-100-3
Polyamine Developer	1 vial	Green	K475-100-4
Sample Clean-Up Mix	1 vial	Blue	K475-100-5
Polyamine Standard	1 vial	Yellow	K475-100-6

V. User Supplied Reagents and Equipment:

- Black 96-well plate with flat bottom
- Multi-well spectrophotometer
- 10 kDa Spin Column (Cat. # 1997 or equivalent)

VI. Storage Conditions and Reagent Preparation:

Store kit at -20°C, protected from light. Briefly centrifuge small vials prior to opening. Read entire protocol before performing the assay.

- **Polyamine Assay Buffer:** Allow to warm to room temperature prior to use. Store at 4°C, protected from light.
- **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 and Polyamine Developer, and Sample Clean-Up Mix:** Reconstitute each vial with 220 µl 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 ddH_2O and mix thoroughly to generate a 100 mM Polyamine Standard solution. Aliquot and store at -20°C. Use within two months.

VII. Polyamine Assay Protocol:

1. **Sample Preparation:** For tissues and cultured cells: add 50 µl of ice-cold Polyamine Assay Buffer per 10 mg of sample (wet weight) or ~1 x 10^6 pelleted cells. Homogenize samples on ice using a Dounce homogenizer (Cat. # 1998). Centrifuge at 10,000 x *g* for 5 min at 4°C. Collect the supernatant. Add 2 µl Sample Clean-Up Mix per 100 µl lysate (or saliva) and incubate for 30 min at RT. Transfer sample to a 10 kD MWCO filter (Cat. # 1997) 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. Adjust the volume to 50 µl per well with Polyamine Assay Buffer. For each sample, prepare identical background control reactions in separate wells.

   Notes:
   - Once treated with Sample Cleanup Mix 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.

2. **Standard Curve Preparation:** Dilute the 100 mM Polyamine Standard by combining 10 µl with 990 µl dH_2O to generate a 1 mM solution. Further dilute the 1 mM solution by adding 50 µl to 950 µl dH_2O, yielding a 50 µM Polyamine Standard working solution. Add 0,

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

3. **Reaction Mix:** Dilute Polyamine Probe 10-fold with anhydrous DMSO (*i.e.* mix 5 µl Polyamine 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. For each test sample well, prepare 50 µl Reaction Mix containing:

	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.

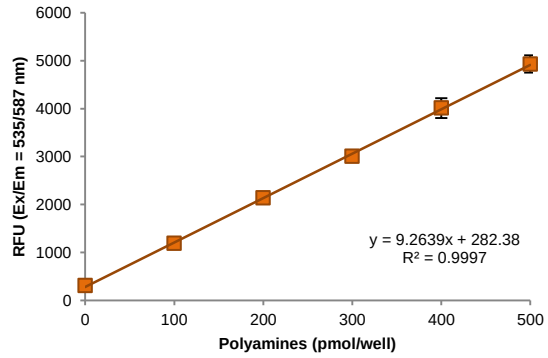
4. **Measurement:** Incubate the plate for 30 min at 37°C, *protected from light* and read the fluorescence (Ex/Em = 535/587 nm) of all reaction, sample background and standard curve wells in endpoint mode.

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

Sample Polyamine Concentration = (*B* / *V*) x *D* = pmol/µl ≡ µM

Where: ***B*** = Polyamine amount from Standard Curve (in pmol)
V = sample volume added into the reaction well (in µl)
D = sample dilution factor (if applicable)

(a)



(b)

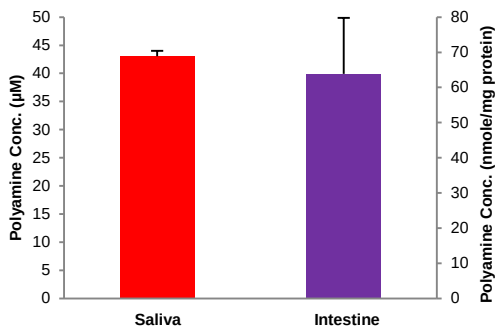


Figure: (a) Polyamine standard curve. **(b)** Determination of total polyamine concentration in saliva (determined to be 43.0 ± 8.9 µM) and intestinal tissue lysate (63.9 ± 16.0 nmole/mg protein). For this experiment, 100 mg rat intestine was homogenized and prepared according to the kit protocol. Saliva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

VIII. RELATED PRODUCTS:

Monoamine Oxidase (Total MAO/MAO-A/MAO-B) Fluorometric Assay Kit (K795)
Monoamine Oxidase A (MAO-A) Inhibitor Screening Kit (Fluorometric) (K796)
Monoamine Oxidase B (MAO-B) Inhibitor Screening Kit (Fluorometric) (K797)
Histamine Assay Kit (Colorimetric) (K506)
Diamine Oxidase Activity Assay Kit (K496)
Histamine (HIS) ELISA Kit (K4163)
Clorgyline Hydrochloride (2622)
Moclobemide (9561)
Pargyline Hydrochloride (2618)

FOR RESEARCH USE ONLY! Not to be used on humans.