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FRONTIERS IN LIFE SCIENCE

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# PolyamineRED™ <Intracellular Polyamine Detection Reagent>

Catalog NO. FDV-0020

Research use only, not for human or animal therapeutic or diagnostic use.

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## Product Background

The polyamine species (Figure 1), including putrescine, spermidine and spermine etc. and its acetyl derivatives, are one of the essential class of metabolites which have liner alkyl structure with two or more amines. Polyamines are found in all living organisms with high concentration, from sub-millimolar to millimolar, in the cells. Polyamines have polycationic properties and shows an enormous number of biological functions. For example, polyamines interact with DNA/RNA in the nuclear and regulate gene expression. Polyamines also interact with negatively charged proteins and control its function. The major source of polyamines is an amino acid ornithine. In the case of mammalian, ornithine is converted to putrescine by ornithine decarboxylase (ODC), followed by synthesizing spermindine and spermine. Because ODC is highly expressed in cancer cells, polyamines are considered as one of the cancer marker. Several detection methods of polyamines are developed to date but most of the methods are commonly low-throughput systems using HPLC with polyamine standard compounds. To clear biological functions of polyamines in the cells, the cell-based assay with easy- and high-throughput-procedures is desired.

**PolyamineRED™** is the world's first reagent for detecting intracellular polyamines without any pre-treatment and cell lysis. PolyamineRED™ is a TAMRA (tetramethylrhodamine)-conjugated derivative of glycine propagyl ester which specifically reacts with linear primary alkylamine but not react with secondary amines, bulky amines including amino acids nor monoamines. PolyamineRED™ has cell-penetrating properties, specifically reacts with polyamines inside the cells and labelled polyamines with red fluorescent dye TAMRA. (Figure 2).

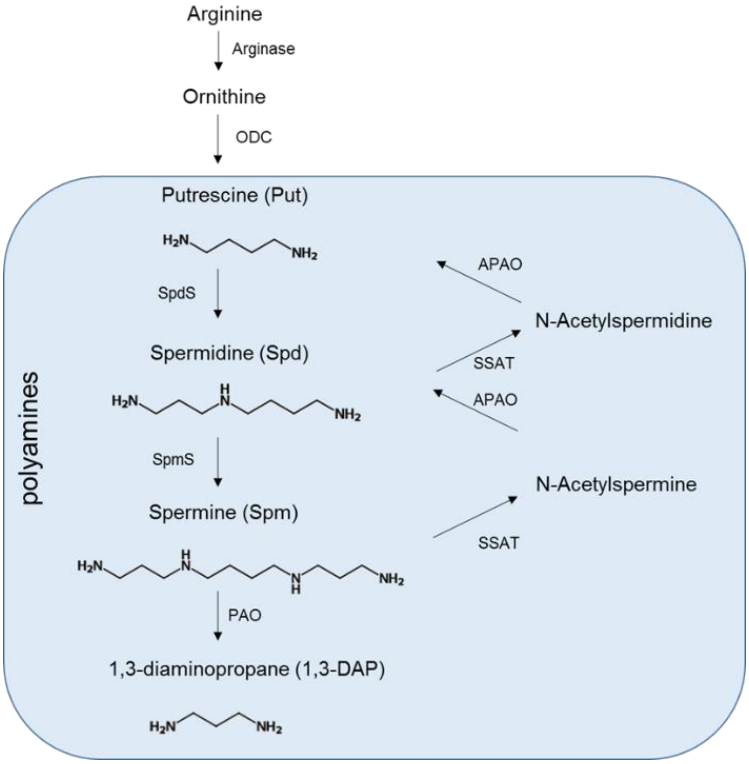



**Figure 1. Major polyamine species**

## Description

Catalog Number: FDV-0020
Size : 0.5 mg
Molecular weight : 611 g/mol
Solubility : Soluble in DMSO
Fluorophore : TAMRA
(red fluorescent dye)
Ex/Em: 560 nm/585 nm
*TAMRA filter sets are available.

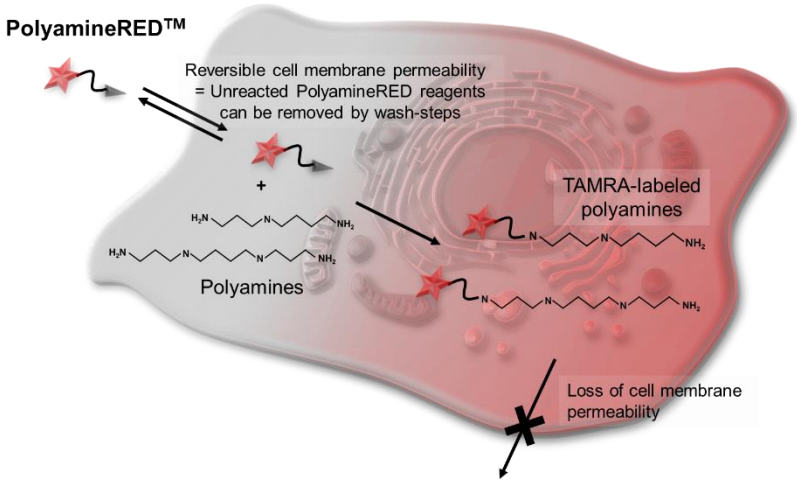



**Figure 2. Principle of PolyamineRED**

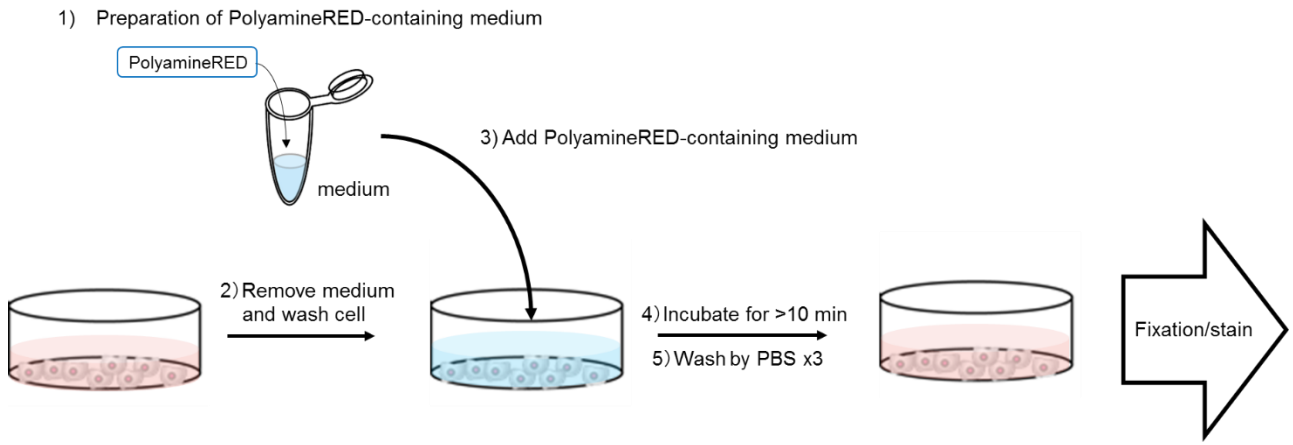
## Reconstitution and Storage

Reconstitution : stock solution in 100% DMSO.
Storage (solution) :
Store powder at -20ºC.
After reconstitution in DMSO, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.
Protect from light.

## How to use

### General procedure of detection of intracellular polyamines

1. Prepare 10-30 µM PolyamineRED™ in fresh medium
2. Remove culture medium, wash cells by PBS twice and add PolyamineRED™ -containing medium to cells
3. Culture cells for at least 10 min
4. Wash cells with PBS 3 times
5. Fixed cells with paraformaldehyde (Option)
   Note: MeOH-fixation is not available. Please fix cells by formaldehyde.
6. Additional staining such as DAPI staining or immunocytochemistry with antibodies of interest are available.



## Reference data

### Selectivity of glycine propagyl ester to polyamines

Benzyloxycarbonyl glycine propagyl ester as a model molecule was selectively reacted with polyamines. Reactant of epinephrine, an example of monoamine, and lysine, an example of amino acid, were rarely detected. Reactivity for polyamines depends on the length of polyamine and double linkage products were observed from spermine (4 amino groups) and spermidine (3 amino groups).

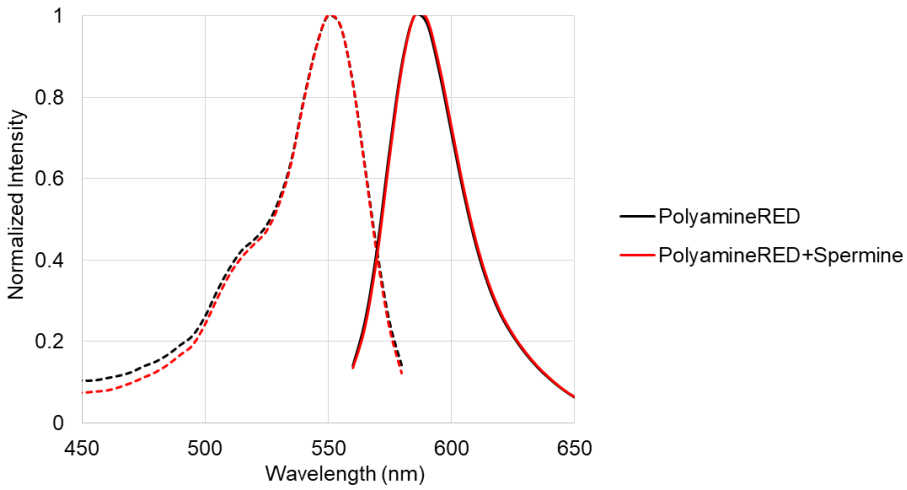
Table Selectivity of glycine propagyl ester to biological amines

| amine | Reaction products | | | Hydrolysis product | Non reacted product |
|---|---|---|---|---|---|
| | Total | Single linkage | Double Linkage | | |
| Spermine | 82% | 59% | 23% | 17% | 1% |
| Spermidine | 78% | 67% | 11% | 21% | 1% |
| Putrescine | 66% | 66% | <1% | 22% | 7% |
| Epinephrine | <1% | <1% | <1% | 7% | 92% |
| Lysine | 2% | 2% | <1% | 6% | 85% |

*This data was cited from Ref.1

### Absorption and Fluorescent spectrum of PolyamineRED™

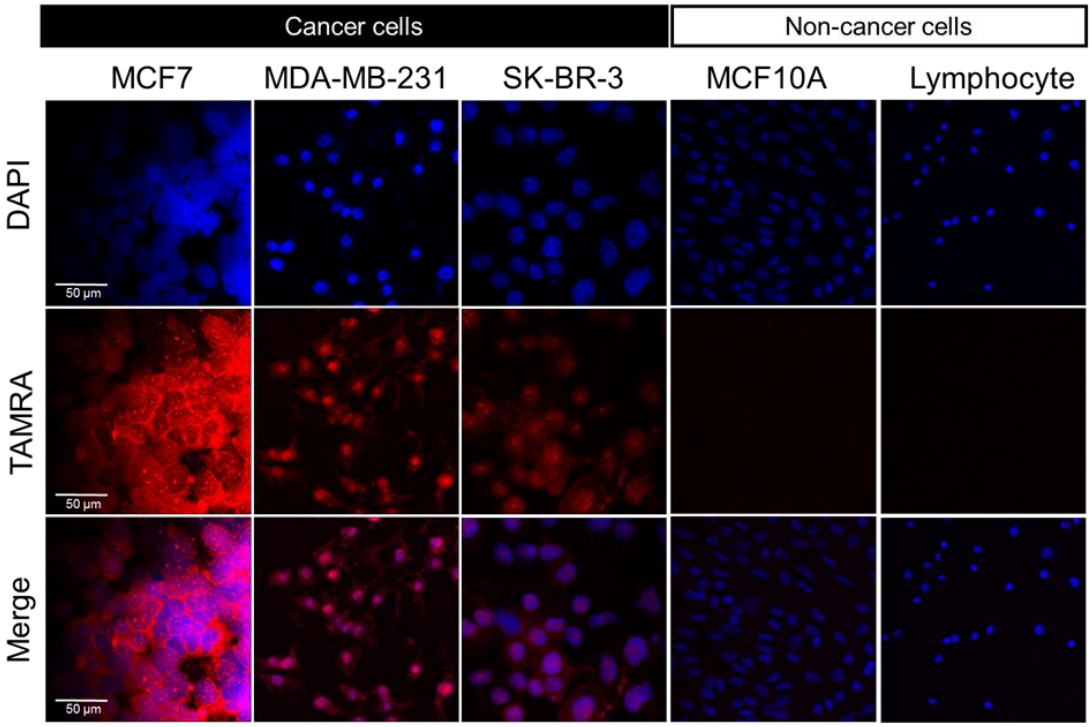
Absorption (dash line) and emission (solid line) of PolyamineRED™ (Black) and PolyamineRED™ with spermine (Red) in PBS.



## Application data

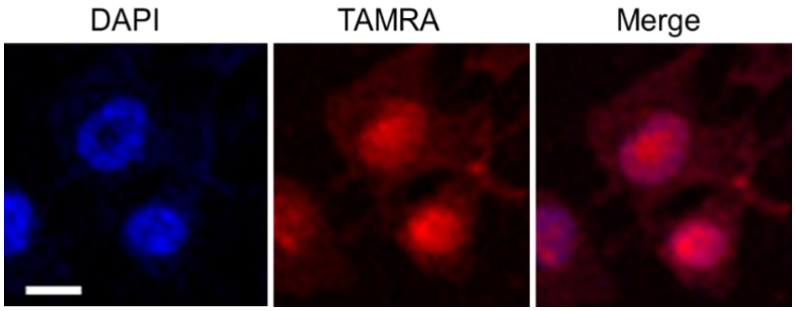
### Polyamine imaging in both cancer and non-cancer cells by PolyamineRED™

Three cancer cell lines (MCF7, MDA-MB-231 and SK-BR-3) and two non-cancer cells (MCF10A and human lymphocyte) were treated with 30 μM of PolyamineRED™ for 10 min. After incubation, cells were washed three times by PBS, followed by DAPI staining and formalin fixation. Images were obtained at Ex/Em=560 nm/585 nm for TAMRA and at Ex/Em=358 nm/461 nm for DAPI. TAMRA fluorescence was detected in cancer cell lines. On the other hand, incubation with non-cancer cell lines showed little fluorescence.



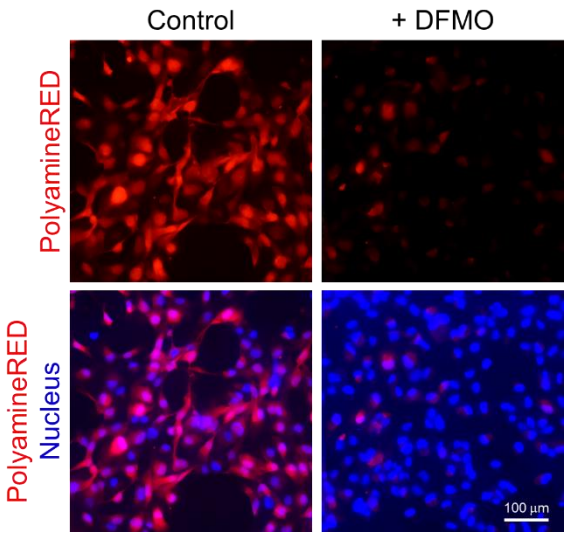

### Evaluation of intracellular distribution of polyamines in MDA-MB-231 cancer cell lines

MDA-MB-231 cells were treated with 30 μM of PolyamineRED™ for 10 min. After incubation, cells were washed three times by PBS, followed by DAPI-staining and formalin fixation. Images were obtained at Ex/Em=560 nm/585 nm for TAMRA and at Ex/Em=358 nm/461 nm for DAPI. Major TAMRA fluorescent signal was detected from nuclear. This indicates polyamines in MDA-MB-231 are mainly localized in nuclear.



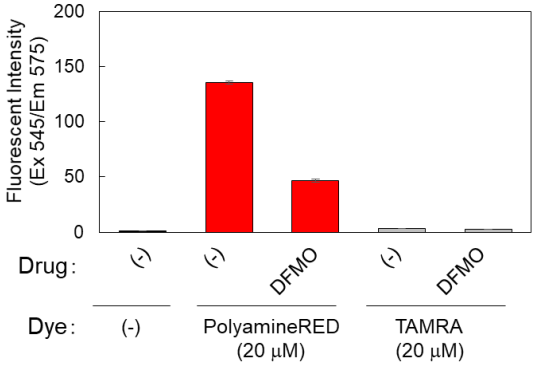
## Evaluation of effect of ODC inhibitor on polyamine biosynthesis in renal cancer cell lines

A renal cancer cell line 786-O cells were seeded in 3.5 cm glass bottom dishes and cultured in 10% FBS/DMEM for 1 day. Then, cells were treated with 1 mM DFMO (an ODC inhibitor) or 1% DMSO (negative control) in serum-free DMEM for 20 hours. After wash cells with HBS twice, the cells were stained with 10 μM PolyamineRED™ and 1 μM Hoechest 33342 in HBS for 1 hour. The cells were washed with HBS 3 times and observed by epi-fluorescent microscopy under live cell condition (Ex.510-560 nm/Em. 590- nm). DFMO-treated cells showed weaker signal of PolyamineRED™ than control cells. This result indicates that DFMO inhibits polyamine biosynthesis.



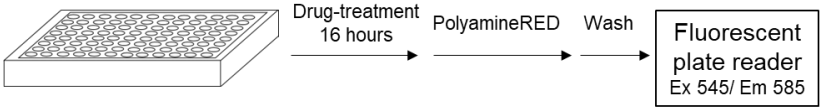

## Detection of PolyamineRED™ signal from cell lysate

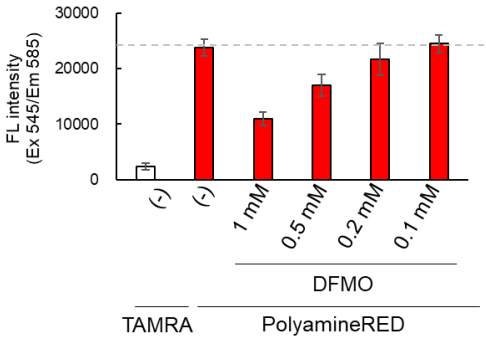
786-O cells were seeded in 24 well and culture for 1 day. Confluent cells were treated with DFMO, a ornithine synthase inhibitor; 1 mM, for 16 hours in phenol red-free DMEM (PRf-DMEM) without FBS and further treated with final 20 μM of PolyamineRED™ or TAMRA as a negative control for 4 hours. Cells were washed by PBS 3 times and added with 1% SDS-containing PBS. Fluorescent intensity (Ex 540±10 nm/ Em 585±20 nm) by the fluorescent spectrophotometer. Fluorescent signals from PolyamineRED™ -treated cells were significantly higher than that of TAMRA negative control experiment. DFMO-treatment reduced fluorescent intensity.



### High-throughput cell-based detection by fluorescent plate leader

786-O cells were seeded in 96 well and culture for 1 day. Confluent cells were treated with indicated drugs for 16 hours in phenol-red free DMEM (PRf-DMEM) without FBS and further treated with final 10 μM of PolyamineRED™ or TAMRA as a negative control for 4 hours. Cells were washed by PBS 3 times and added with fresh PBS. Fluorescent intensity (Ex 545±5 / Em 585±10) by the fluorescent plate leader with a transparent mode. Fluorescent signals were normalized by background signal of wells of non-dye treated cells. Fluorescent signals from PolyamineRED™ -treated cells were significantly higher than the TAMRA negative control experiment.




## Reference

1. K. Vong, K. Tsubokura, Y. Nakao, T. Tanei, S. Noguchi, S. Kitazume, N. Taniguchi, K. Tanaka, *Chem. Commun.*, **52**, 8403 (2017). Cancer cell targeting driven by selective polyamine reactivity with glycine propargyl esters.

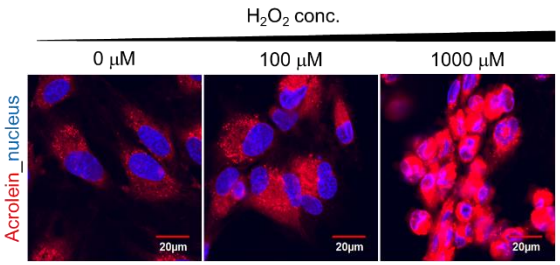
## Related product

### AcroleinRED™ <Cell-based Acrolein Detection Reagent>

Acrolein is one of the most toxic oxidative stress marker and AcroleinRED™ is the world first cell-based acrolein detection reagent. As polyamines are one of the major source of acrolein, AcroleinRED™ and PolyamineRED™ are good set for oxidative stress research.

Catalog No. FDV-0022
Size 0.5 mg
Features

- Recommended Ex/Em: 560 nm/585 nm
- Easy and quick protocol
- Enable to semi-quantify intracellular acrolein

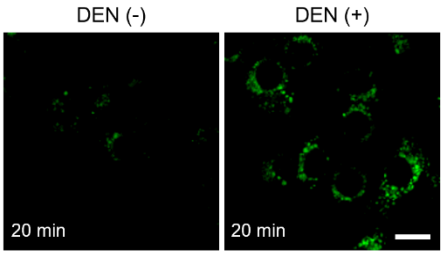



### LipiRADICAL™ Green <Lipid Radical Detection Reagent>

LipiRADICAL™ Green is a specific fluorescent dye for lipid-derived radicals which are the most upstream factor of lipid peroxidation (LPO). LipiRADICAL™ Green can be applied into both *in vitro* assay and cell-based assay to monitor lipid radical productions.

Catalog No. FDV-0042
Size 0.1 mg
Features

- Recommended Ex/Em:~480 nm / 520 nm
- Enable to detect very unstable lipid-derived radicals
- Compatible with *in vitro* assay and in cell-based assay
- An innovative reagent for comprehensive identification of lipid-derived radicals by lipidomics

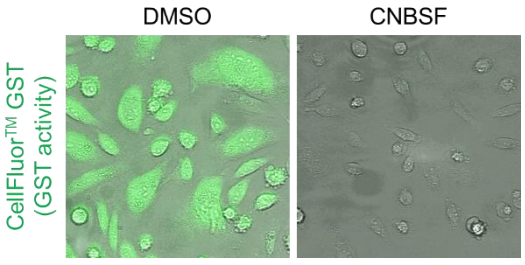



### CellFluor™ GST <Cell-based GST Activity Assay Reagent >

CellFluor™ GST is a novel fluorescent probe for monitoring wide GST members' activity both *in celluo* or *in vitro*. CellFluor™ GST releases green fluorophore rhodamine 110 upon GST activities. This probe has cell-permeability and can detect intracellular GST activity.

Catalog No. FDV-0031
Size 0.1 μmol
Features

- Easy and quick protocol
- Broad specificity for various GST family members
- Ex/Em: 496 nm/520 nm
  (Compatible with commercial FITC filters)



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