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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 use in human.

This product has been commercialized with the support of Biofunctional Synthetic Chemistry Laboratory, RIKEN.

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 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).

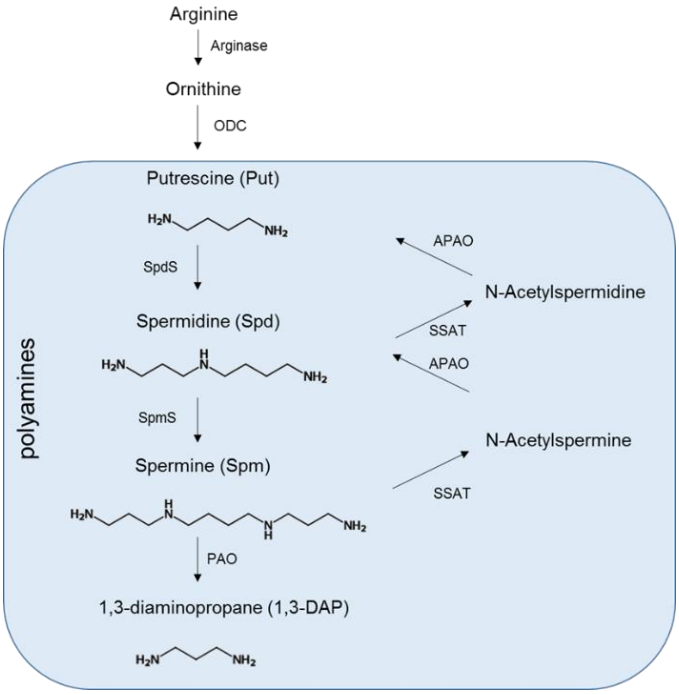


Figure 1. Major polyamine species

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
*Rhodamine filter sets are available.

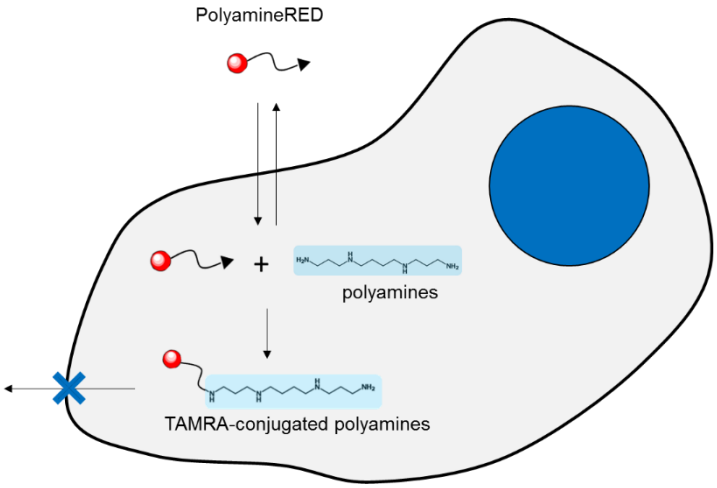


Figure 2. Principle of PolyamineRED

Reconstitution and Storage

Reconstitution : stock solution in 100% DMSO.
Storage (solution) :
Store powder at -20°C.
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
   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.

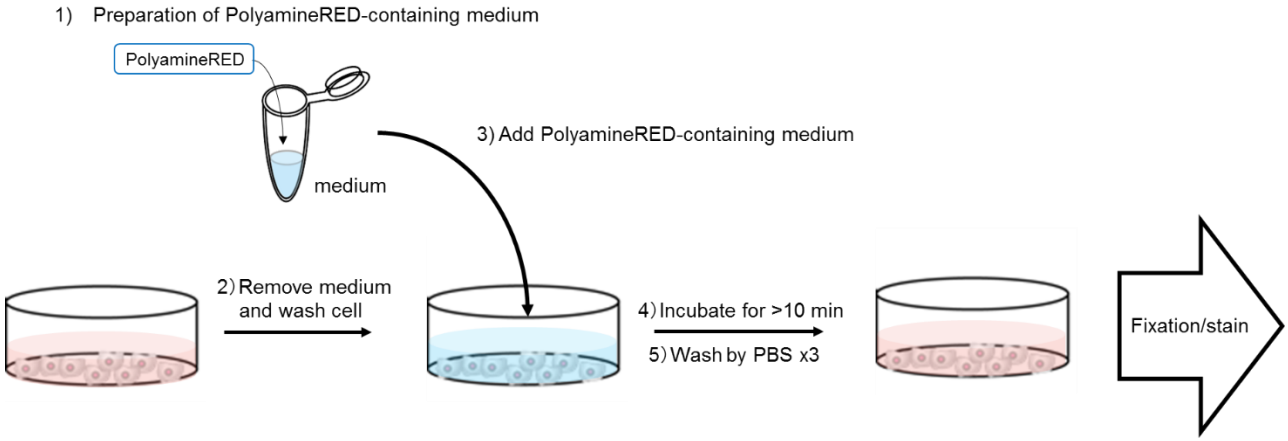
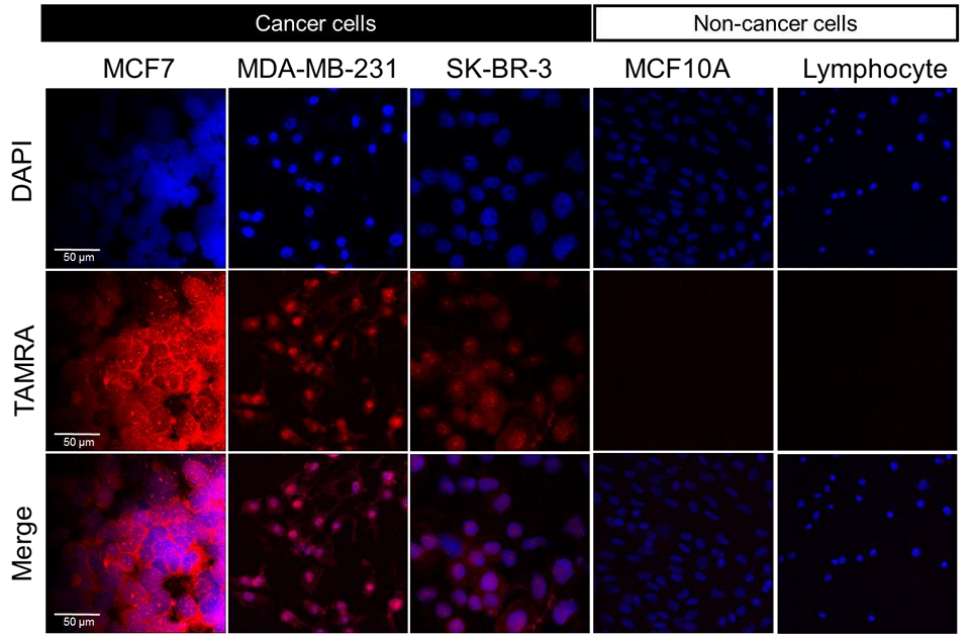


Figure 3. Overview of experimental procedure

Application data

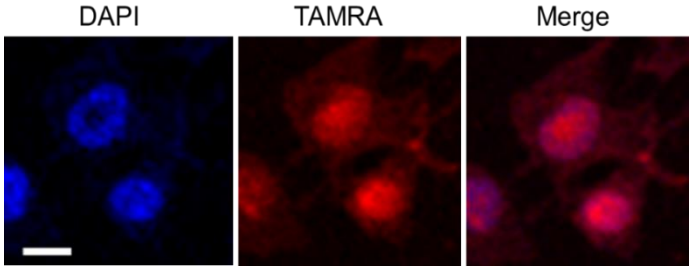
Polyamine imaging in both cancer and non-cancer cells by PolyamineRED

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.



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.

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

Related product

AcroleinRED <Cell-based Acrolein Detection Reagent>

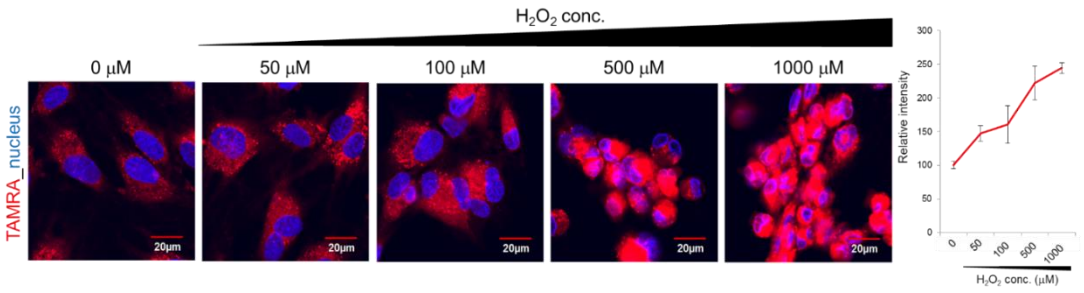
Acrolein is one of the most toxic oxidative stress marker and AcroleinRED is the world first cell-based acrolein detection reagent. As polyamines are one of the major source of acrolein, AcroleinRED and PolyamineRED are good set for oxidative stress research.

Catalog No. FDV-0022
Size 0.5 mg

Data example

Observation of oxidative stress-induced acrolein production

HUVECs were pretreated with 0-1000 μM H_2O_2 for 2 hours and subsequently treated with 10 μM AcroleinRED for 30 min. Right after labeling, cells were washed, stained with hoechest and observed under live cell condition. In the absence of H_2O_2, the acrolein endogenously produced by HUVECs could be observed. Intracellular TAMRA signals were increased in the H_2O_2 dose-dependent manner compared with the endogenous acrolein level.



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