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FRONTIERS IN LIFE SCIENCE

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AcroleinRED <Cell-based Acrolein Detection Reagent>

Catalog NO. FDV-0022

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

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

Product Background

Acrolein, the simplest α,β-unsaturated aldehyde (chemical name; 2-propenal), is highly toxic metabolite for cells. Acrolein is found in a common dietary and environmental pollutant such as tobacco smoke. Acrolein is also endogenously generated in cells, especially under the oxidative stress condition. Enzymatic oxidation of threonine and polyamine species, reactive oxygen species (ROS)-mediated oxidation of highly unsaturated lipids via lipid peroxidation (LPO) pathway are major source of acrolein in cells. Recent evidences indicate acrolein is more toxic to cells than ROS such as hydrogen peroxide (H_2O_2) or hydroxyl radicals. Because of its highly reactivity, acrolein in the cell immediately reacts with various biomolecules such as DNA/RNA and proteins, and subsequently impairs their functions.

Establishment of the detection methods of cellular acrolein is an important issue to understand physiological relevance of acrolein. **AcroleinRED** is the world first cell-based acrolein-detection reagent under the live cell condition without any pre-treatment and cell lysis. This reagent is based on phenylazide-acrolein click chemistry discovered by Drs. Katsunori Tanaka and Ambara Pradipta (Ref.1). AcroleinRED specifically react with either extracellular acrolein released from cell surface lipids or intracellular acrolein generated via enzymatic pathway and label acrolein with TAMRA fluorophore. In the case of extracellular labeling, TAMRA-labeled acrolein are immediately incorporated into cells through the endocytosis pathway. The TAMRA-labeled acrolein react with biomolecules such as a protein and stay in the cells (Figure 1).

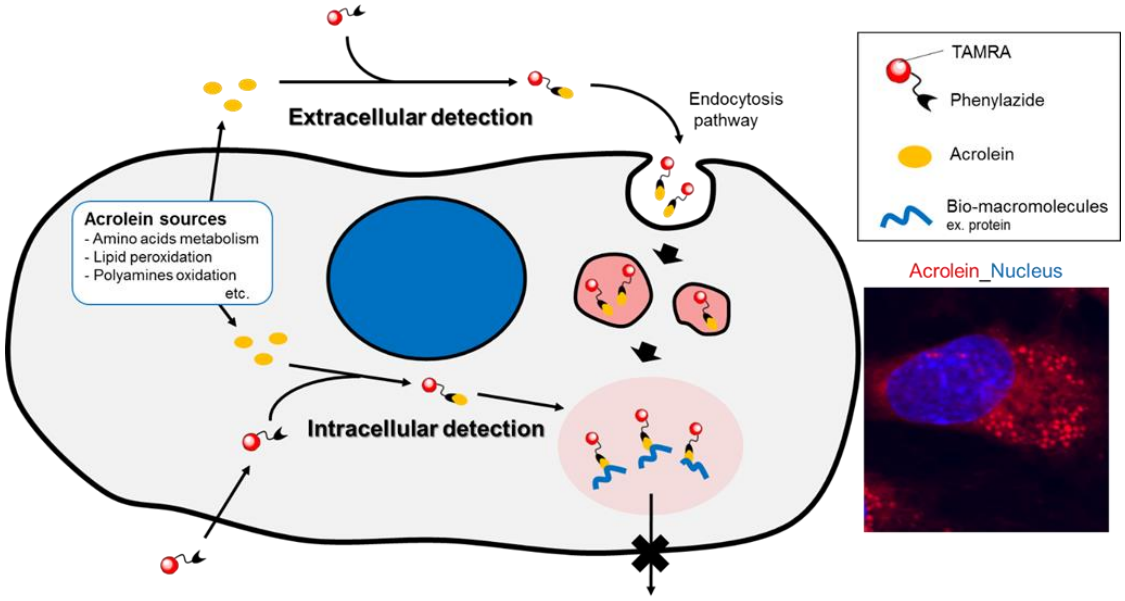


Figure 1. Principle of detection of acrolein

Description

Catalog Number: FDV-0022
Size : 0.5 mg
Molecular weight : 560 g/mol
Solubility : Soluble in DMSO
Fluorophore : TAMRA (red fluorescent dye)
Ex/Em: 560 nm/585 nm
*TAMRA filter sets are available.

Application

- Semi-quantification of cellular acrolein
- Estimation of effects of any drugs on cellular acrolein production
- Staining of *ex vivo* tissues under non-fixed condition

NOTE: AcroleinRED cannot be applied into localization study of intracellular acrolein.

Reconstitution and Storage

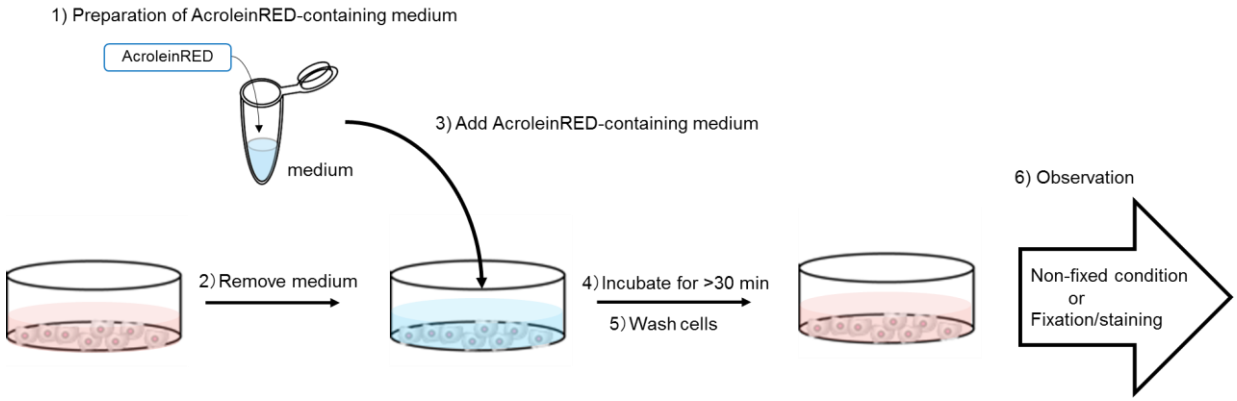
Reconstitution : stock solution in 100% DMSO.
Storage (solution) :
Store powder at -20ºC.
After reconstitution in DMSO, aliquot and store at -20 °C. Avoid repeated freeze-thaw cycles.
Protect from light.

How to use

General procedure of detection of cellular acrolein

1. Prepare 10-30 μM AcroleinRED in fresh medium
   NOTE: Please optimize concentration of reagent for your experiments.
2. Remove culture medium
3. Add AcroleinRED-containing medium to cells
4. Incubate cells for 30-60 min
5. Wash cells with PBS or medium 3 times
6. Observe cells in either non-fixed or fixed cells with paraformaldehyde

Option: Additional staining such as nuclear staining or immunocytochemistry with antibodies of interest are available



Reference data

Selectivity of phenylazide to acrolein

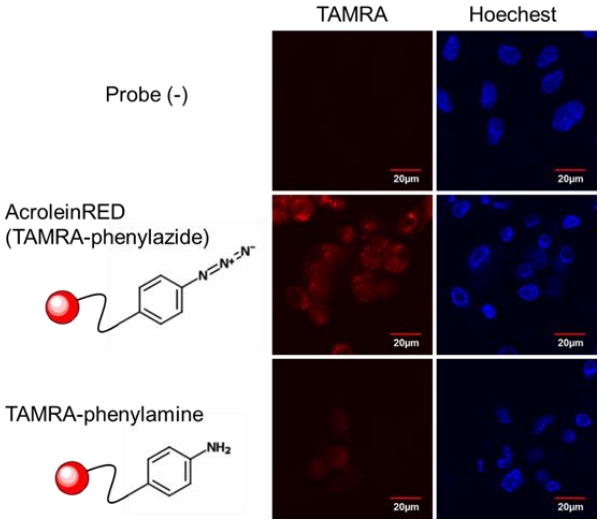
Detection of AcroleinRED is based on phenylazide-acrolein click reaction. Reaction efficiencies of phenylazide to acrolein, acrolein derivatives, and the other unsaturated molecules were listed in **Table**.

Table Specificity of phenylazide

Phenylazide with	Reaction (%)
Acrolein	**47**
Methacrolein	<1
Crotonaldehyde	<1
Trans-2-octenal	<1
Styrene	<1

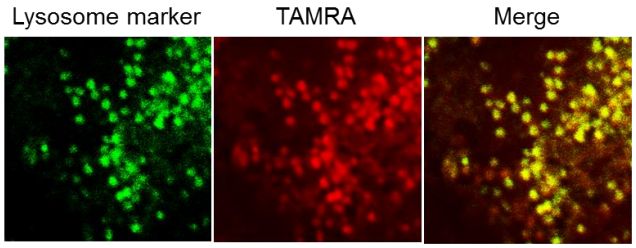
AcroleinRED specifically detects cellular acrolein.

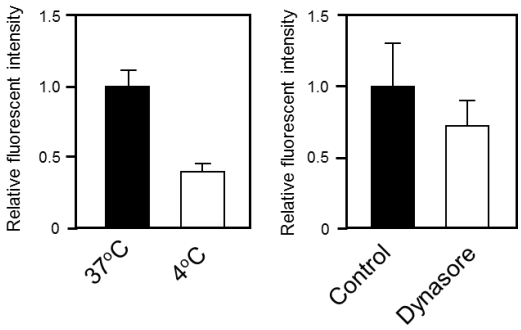
AcroleinRED (TAMRA-conjugated phenylazide) and its similar structure, TAMRA-conjugated phenylamine, were added into HUVECs at 10 μM for 30 min. After incubation, cells were fixed and also stained by Hoechest. Only AcroleinRED-treated cells showed red fluorescent signal.



Endocytosis-dependent uptake of AcroleinRED-acrolein conjugates

HUVECs were treated with the synthesized AcroleinRED-acrolein conjugates for 30 min in the presence of lysosome marker. The TAMRA signals from AcroleinRED-acrolein conjugate were well corresponding with lysosome marker (Left panel). Furthermore, cellular uptake of AcroleinRED-acrolein conjugate clearly suppressed at lower temperature or in the presence of an endocytosis blocker, Dynasore (Right panels). These results suggest uptake of AcroleinRED-acrolein conjugate depends on endocytosis pathway and AcroleinRED-acrolein conjugates localized in lysosomes.



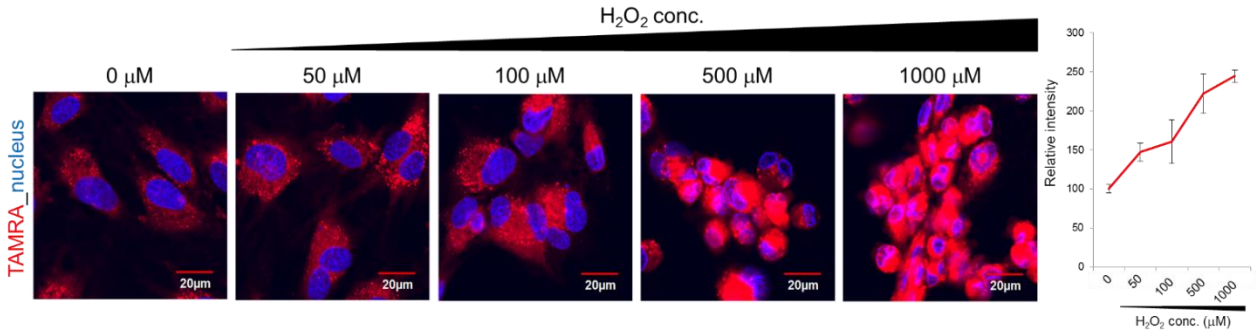


*All data are cited from Ref.1

Application examples

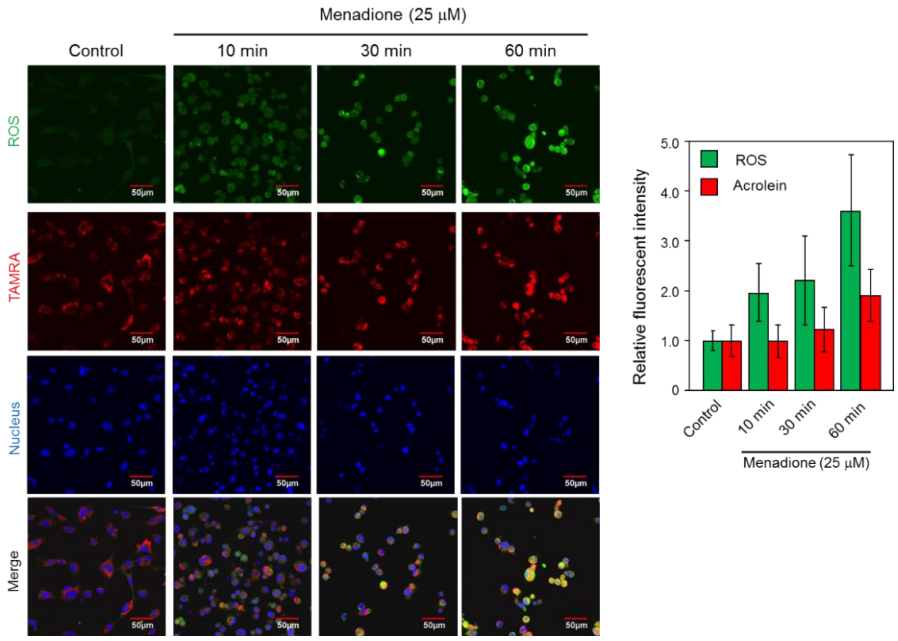
Observation of oxidative stress-induced acrolein production

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.
Note: Ref.1 confirmed AcroleinRED did not react with H_2O_2 directly. Please refer Ref.1 for the detail experiments.



Observation of reactive oxygen species (ROS)-induced acrolein production

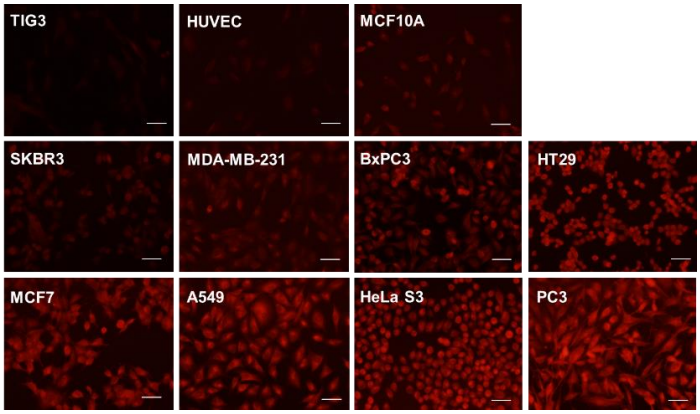
HUVEC were treated with 25 μM menadione, an inducer of reactive oxygen species, for 0-60 min and subsequently treated with Total ROS detection dye (Enzo Life Science) and AcroleinRED for 60 min. After labeling, fluorescent signal of ROS (green) or acrolein (red) were observed. By the addition of menadione, ROS were immediately increased, however, the acrolein level started to increase 60 min after menadione treatment. Namely, the late stage production of acrolein through ROS-initiated process was clearly imaged by using AcroleinRED.

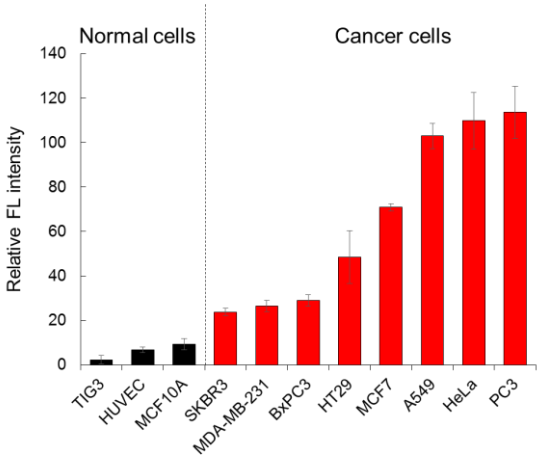


*All data above are cited from Ref.1

Comparison of acrolein-production levels of various cell lines by AcroleinRED

Three non-cancer cells (TIG4, HUVEC and MCF10A) and eight cancer cell lines were treated with 22.5 μM of AcroleinRED for 30 min at 37°C. Red fluorescent signals of cancer cells were much higher than that of normal cells. Furthermore, AcroleinRED revealed that acrolein-production levels of eight cancer cell lines are significantly different from each other.

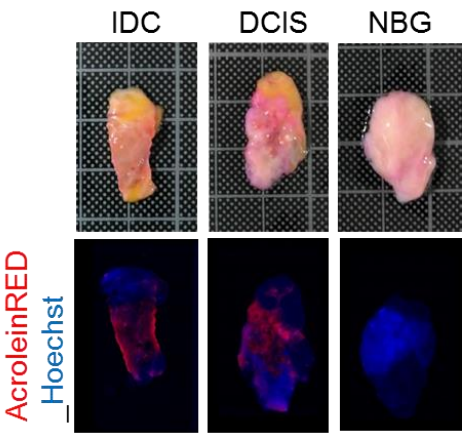




Visualization of tumors from breast gland tissues

Surgical tissues derived from breast cancer patients or a healthy control were immediately incubated with AcroleinRED (20 μM)/Hoechst mixed solution under non-fixed condition for 5 min. After washing tissues by PBS, double-stained tissues were observed by fluorescent microscopy. AcroleinRED visualized tumor region of patient-derived breast gland, but not derived from healthy control.

IDC; invasive ductal carcinoma, DCIS; ductal carcinoma in situ, NBG; normal breast gland



* Above two data are cited from 2

Reference

1. A.R. Paradipt, M. Taichi, I. Nakase, E. Saigitbatalova, A. Kurbangalieva, S. Kitazume, N. Taniguchi, K. Tanaka, *ACS Sens.*, **1**, 623-632 (2016) Uncatalyzed click reaction between phenyl azides and acrolein: 4-formyl-1,2,3-triazolines as “clicked” markers for visualizations of extracellular acrolein released from oxidatively stressed cells.
2. T. Tanei, A. R. Pradipta, K. Morimoto, M. Fujii, M. Arata, A. Ito, M. Yoshida, E. Saigitbatalova, A. Kurbangalieva, J. Ikeda, E. Morii, S. Noguchi, and K. Tanaka, *Adv. Sci.*, 1801479 (2018) Cascade reaction in human live tissue allows clinically applicable diagnosis of breast cancer morphology.

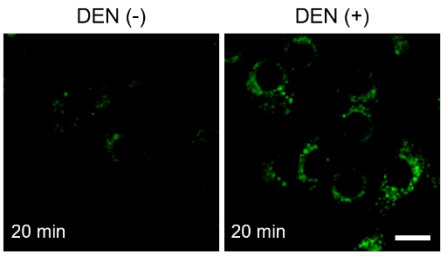
Related products

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

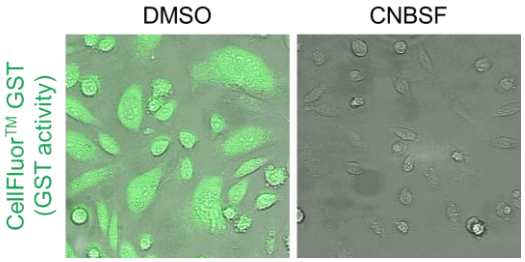


CellFluor™ GST <Cell-based GST Activity Assay Reagent >

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-0030
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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