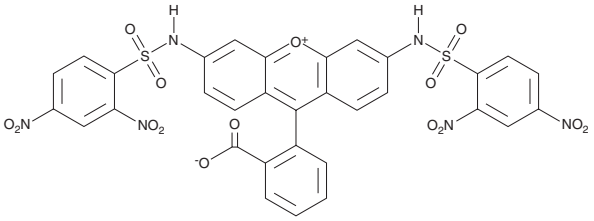
PRODUCT INFORMATION

DNs-Rh

Item No. 42354

CAS Registry No.:	1028386-82-4
Formal Name:	9-(2-carboxyphenyl)-3,6-*bis*[[(2,4-dinitrophenyl)sulfonyl]amino]-xanthylium, inner salt
Synonym:	*bis*-DNs-Rh
MF:	$C_{32}H_{18}N_6O_{15}S_2$
FW:	790.6
Purity:	≥95%
Ex./Em. Max:	496/520 nm
Supplied as:	A solid
Storage:	-20°C
Stability:	≥4 years



Information represents the product specifications. Batch specific analytical results are provided on each certificate of analysis.

Laboratory Procedures

DNs-Rh is supplied as a solid. A stock solution may be made by dissolving the DNs-Rh in the solvent of choice, which should be purged with an inert gas. DNs-Rh is soluble in DMSO.

Description

DNs-Rh is a cell-permeable quenched fluorescent probe for the detection of pan-glutathione S-transferase (GST) activity in adherent or suspension cells, cell or tissue lysates, or purified enzyme preparations. It is composed of rhodamine 110 (Item No. 19061) protected by the GST substrate dinitrobenzenesulfonamide (DNB).[1,2] Upon deprotection by GSTs, rhodamine 110 is released, and its fluorescence can be quantified using excitation/emission maxima of 496/520 nm, respectively.

Protocol for Measuring Intracellular GST Activity

1. Prepare a working solution of DNs-Rh at a concentration of 1-30 µM in serum- and phenol red-free medium or buffer, such as HEPES-buffered saline (HBS; 20 mM HEPES, pH 7.4, 107 mM NaCl, 6 mM KCl, 1.2 $MgSO_4$, and 2 mM $CaCl_2$). *NOTE: Optimize the concentrations of* DNs-Rh, *culture medium, or buffer, as needed.*
2. Remove culture medium from cells.
3. Add DNs-Rh working solution.
4. Incubate cells for at least 10 minutes and measure fluorescence intensity. For fluorescence intensity quantification using a plate reader or flow cytometry, do not wash the cells. For fluorescence intensity quantification using fluorescence microscopy, wash the cells using a buffer, such as PBS, and image immediately.

Protocol for Measuring GST Activity in Cell-Free Preparations

1. Prepare a solution containing DNs-Rh at a concentration of 1-10 µM, glutathione (GSH) at a concentration of 10-100 µM, and GST-containing samples, such as cell or tissue lysates, purified enzymes, *etc. NOTE: The assay buffer used for the samples must be optimized for your experimental conditions.*
2. Preparing a negative control containing DNs-Rh and GSH without GST-containing samples is highly recommended.
3. Incubate for 30-60 minutes and measure fluorescence intensity.
   NOTE: An incubation of greater than 60 minutes is not recommended. DNs-Rh interacts with free thiol groups, including in GSH, albeit at a lower reaction efficiency than with GSTs. Due to the requirement of exogenous GSH in the cell-free GST activity assay, background fluorescence may be

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observed under long-term incubation times.

References

1. Shibata, A., Furukawa, K., Abe, H., *et al.* Rhodamine-based fluorogenic probe for imaging biological thiol. *Bioorg. Med. Chem. Lett.* **18(7),** 2246-2249 (2008).
2. Ålander, J., Johansson, K., Heuser, V.D., *et al.* Characterization of a new fluorogenic substrate for microsomal glutathione transferase 1. *Anal. Biochem.* **390(1),** 52-56 (2009).

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