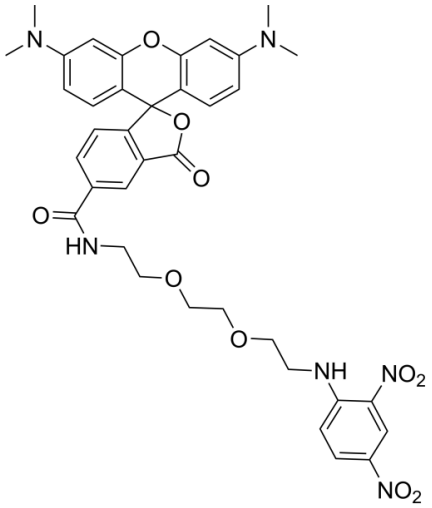
Product Data Sheet

Product Name: TMR-DN
Cat. No.: GC80353

Chemical Properties

Cas. No.			
Formula	$C_{37}H_{38}N_6O_{10}$	M.Wt	726.73
Solubility		Storage	Store at -20°C
General tips	For obtaining a higher solubility , please warm the tube at 37 °C and shake it in the ultrasonic bath for a while.Stock solution can be stored below -20°C for several months.		
Shipping Condition	Evaluation sample solution : ship with blue ice All other available size: ship with RT , or blue ice upon request.		

Structure



Background

TMR-DN (TAMRA-2,4-dinitroaniline) is a fluorescent probe for RNA imaging that consists of a covalently coupled fluorophore (5-carboxy tetramethylrhodamine (TMR)) and quencher (dinitroaniline (DN)). The K d value of TMR-DN with SRB-2 is 35 nM, and its K d value with RhoBAST is approximately 30 nM. TMR-DN has a contact quenching function and forms a non-fluorescent intramolecular ground-state dimer in the free state; the excitation/emission wavelengths of the free probe are Ex/Em = 557/579 nm, those of the complex formed by binding to SRB-2 are Ex/Em = 561/587 nm, and those of the probe bound to the DNB aptamer are Ex/Em = 555/582 nm. TMR-DN

reduces non-specific binding to genomic nucleic acids via negatively charged carboxyl-functionalized aromatic rings. Fluorescence dequenching occurs when TMR-DN specifically binds to SRB-2, RhoBAST or the DNB aptamer, among which the binding of RhoBAST requires Mg 2+ for proper folding [1] [2] [3].

In Vitro, Guide (The following is our recommended solution. This solution is merely a guideline and should be modified according to your specific needs.) 1. Stock Solution Preparation 1.1 Solvents: DMSO or DMF. 2. Working Solution Preparation 2.1 Diluents: Live cell imaging solution (for bacterial samples); FluoroBrite DMEM medium supplemented with 2 mM GlutaMAX (for HeLa cell samples); 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 10 mM MgCl2 (for RNA samples); 40 mM Tris, 5 mM MgCl2, 100 mM KCl (pH 7.6) (for in vitro experiments); DPBS (for cell imaging). 2.2 Working concentrations: 0.1 μM, 0.25 μM, 0.5 μM, 1 μM, 2 μM. 2.3 Notes: Adjust the working solution concentration as needed; prepare fresh before use. 3. Staining Protocol 3.1 Sample Type Description 3.1.1 Adherent cells (HeLa cells)[1]: Seed onto chamber coverslips, with cell confluency reaching 60%-80% before transfection. 3.1.2 Bacteria expressing aptamers (e.g., DNB/SRB-2)[1][3]: If an inducible promoter is used, add 1 mM IPTG and continue shaking culture at 37°C for 2-3 hours. Take 200 μL of bacterial culture, collect the bacterial cells by centrifugation, and resuspend them in 1 mL of imaging buffer (containing 5 mM MgSO4). 3.1.3 Purified RNA samples (RhoBAST aptamer and its mutants)[2]. 3.2 Incubation Conditions 3.2.1 For adherent HeLa cells[1]: Incubate with 0.5 μM TMR-DN at 37°C for approximately 30 min. 3.2.2 For suspended bacterial cells[1][3]: Add an equal volume of dye working solution to achieve a final concentration of 0.5-1 μM, and incubate at 37°C for approximately 10 min. 3.2.3 For purified RNA samples (RhoBAST aptamer and its mutants)[2]: Incubate with 0.5 μM TMR-DN in a buffer containing 20 mM Tris-HCl (pH 7.5), 100 mM KCl, 10 mM MgCl2. 3.2.4 For in vitro experiments[3]: Incubate with 0.5 μM TMR-DN, together with RNA constructs and target analytes (if applicable), in a buffer containing 40 mM Tris, 5 mM MgCl2, 100 mM KCl (pH 7.6). 3.3 Washing Steps 3.3.1 Since free TMR-DN shows extremely low background, washing is generally not required after incubation, and imaging can be performed directly. If background is relatively high, gently wash 1-2 times with pre-warmed buffer. 4. Control Setup 4.1 Negative Controls 4.1.1 For bacterial experiments[1][3]: Use bacteria without target-binding aptamers[3]. 4.1.2 For HeLa cell experiments[1]: Use cells without target-binding aptamers. 4.1.3 For in vitro experiments [3]: Set up experiments without target analytes. 4.2 Positive Controls[3]: Cells expressing target-binding

ratiometric sensors with known target concentrations; in vitro experiments supplemented with target analytes of known concentrations. 4.3 Blank Controls[3]: In vitro experiments containing only TMR-DN (without RNA aptamers). 5. Detection and Analysis 5.1 Instrument Types: Fluorescence microscope; multi-mode microplate reader; fluorescence spectrophotometer; confocal microscope, etc. 5.2 Excitation/Emission Wavelengths: 560/40 nm excitation, 630/60 nm emission; 555 nm excitation, 582 nm emission. 5.3 Result Analysis 5.3.1 Changes in fluorescence intensity: The fluorescence turn-on fold of TMR-DN after binding to SRB-2 is approximately 17-fold; the fluorescence intensity of cells expressing SRB-2 is significantly higher than that of negative control cells[1]; the fluorescence intensity of TMR-DN increases after binding to RhoBAST [2]; the fluorescence of free TMR-DN is quenched, and gets activated after binding to DNB aptamer; the fluorescence intensity is further enhanced when the target analyte binds to the ratiometric sensor[3]; non-target analogs only induce extremely weak fluorescence activation, confirming the specificity of the sensor[3]. 5.3.2 Fluorescence localization: Orange fluorescence localizes to cellular regions where SRB-2-tagged RNA is present, including bacterial cytoplasm/poles, HeLa cell nuclei and cytoplasm, as well as HeLa cell cytoplasm where membrane-targeted mRNA resides; whole-cell distributed fluorescence is observed in bacterial cells, and target analytes may show polar aggregation. 5.3.3 Fluorescence color: Orange fluorescence is produced after binding to SRB-2[1]; red fluorescence is produced after binding to DNB aptamer[3]. 5.3.4 Molecular interaction analysis: TMR-DN binds to the semi-open pocket at the end of the asymmetric "A"-shaped structure of RhoBAST, with the xanthene group of TMR inserting into the binding core, and the DN quencher stacking with the benzene ring of TMR[2].

References:

[1]. Sunbul M, et al. SRB-2: a promiscuous rainbow aptamer for live-cell RNA imaging. Nucleic acids research. 2018 Oct 12;46(18):e110.
[2]. Zhang Y, et al. Structural mechanisms for binding and activation of a contact-quenched fluorophore by RhoBAST. Nature communications. 2024 May 17;15(1):4206.
[3]. Wu R, et al. Ratiometric Fluorogenic RNA-Based Sensors for Imaging Live-Cell Dynamics of Small Molecules. ACS applied bio materials. 2020 May 18;3(5):2633-2642.

Caution: Product has not been fully validated for medical applications. For research use only.
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