

Staining Fixed Cells with TOR-G4

G-quadruplexes (G4s) are secondary structures that form in DNA and RNA through noncanonical hydrogen bonding between four guanine bases^[1,2]. In the nucleus, DNA G4s have been associated with epigenetic regulation of gene expression through their interactions with regulatory proteins, such as transcription factors and chromatin modifiers^[3,4]. RNA G4s have been linked to RNA splicing, transport, and translation regulation, as well as RNA-mediated stress responses in the cytoplasm^[5-7].

TOR-G4 is a thiazole orange derivative, a newly synthesized G4 fluorescent probe^[8]. It is a small-molecule alternative to immunochemistry with G4-specific antibodies. TOR-G4 allows the visualization of G4s based on changes to the fluorescence lifetime of a probe upon nucleic acid binding. The lifetime of TOR-G4 is highest in the presence of G4s and lower for other sequences. Within cells, TOR-G4 is primarily colocalized with RNA in the cytoplasm and nucleoli, making it the first lifetime-based probe validated for exploring the emerging roles of RNA G4s in cell functioning. TOR-G4 is suitable for imaging RNA G4s via FLIM (Fluorescence Lifetime Imaging Microscopy)^[8].

Protocol

Important! TOR-G4 is a cytotoxic compound with a GI50 concentration of 143 nM and is intended for staining only fixed cells.

1. Culture cells on an appropriate imaging dish.
2. Wash cells three times with ice-cold PBS before fixation.
3. Fix cells with 4% paraformaldehyde for 10 min at 4 °C.
4. Wash cells three times with ice-cold PBS before staining.
5. Incubate fixed cells with TOR-G4 (5 µM in PBS) for 2 h. The exact concentration and incubation time depend on cell type.
6. Image cells without preliminary washing under a confocal microscope (excitation at 467 nm and detection at 540 nm).
Additionally, FLIM can be utilized to measure the specific fluorescence lifetime changes associated with G4 binding (excitation at 483 nm and emission detection at 650 nm when bound to G4s).

^[1] Trends in Chemistry 2, 123 (2020); ^[2] Nat. Rev Mol Cell Biol 21, 459 (2020); ^[3] Nucleic Acids Res 49, 8419 (2021); ^[4] Trends Genet 35, 29 (2019); ^[5] Nucleic Acids Res 48, 12534 (2020); ^[6] Trends Biochem Sci 46, 270 (2021); ^[7] Nucleic Acids Res 49, 5426 (2021); ^[8] J Am Chem Soc 146, 1009 (2024).

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