

## Product Data Sheet

Product Name: HBC599  
Cat. No.: GC63549

### Chemical Properties

Cas. No. 2530162-00-4

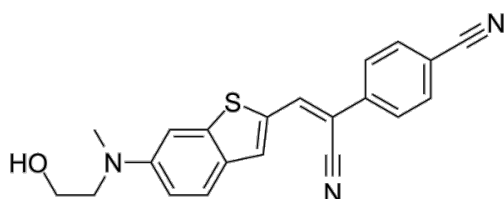
Formula  $C_{21}H_{17}N_3OS$  M.Wt 359.44

Solubility DMSO :  $\geq 25$  mg/mL (69.55 mM) Storage Store at 2-8°C, protect from light

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



### Protocol

#### Staining with HBC599 for Live-Cell RNA Imaging

This protocol is adapted from research data and provided for reference only. Adjustments may be necessary based on specific experimental requirements.

(1) Cell Preparation and Transfection: Seed appropriate cells (e.g., HeLa, U2OS) onto glass-bottom dishes or coverslips and culture until they reach 50-70% confluency. Transfect the cells with a plasmid or RNA expressing the RNA of interest tagged with the Pepper RNA aptamer sequence. Incubate for 12-48 hours to allow for sufficient RNA expression.

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

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(2) HBC599 Solution Preparation: Prepare a 1-5mM stock solution of HBC599 using anhydrous DMSO. Aliquot and store at -20°C or -80°C protected from light. Dilute the HBC599 stock solution in pre-warmed, serum-free cell culture medium (e.g., DMEM or Opti-MEM) to achieve a final working concentration typically between 0.5 to 5µM. Mix thoroughly.

(3) Cell Staining: Gently wash the transfected cells 1-2 times with warm, serum-free medium or PBS. Completely remove the wash buffer and add the prepared HBC599 staining medium to the cells. Incubate the cells in the staining medium at 37°C, 5% CO<sub>2</sub> for 15 to 30 minutes. Carefully aspirate the staining solution. Wash the cells 2-3 times with warm, serum-free medium or 1X PBS.

(4) Live-Cell Imaging: After the final wash, add a small volume of pre-warmed, fresh culture medium to the cells. Proceed to image the cells immediately on a confocal or epifluorescence microscope. HBC599 fluorescence is typically excited with a 515nm laser and detected using a 599nm emission filter.

### Precautions:

(1) The incubation time can range from 30 to 90 minutes with minimal impact on result accuracy; choosing 1 hour is most convenient.

(2) For your safety and health, please wear a lab coat and disposable gloves during operation.

### References:

[1] Rees HC, Gogacz W, Li NS, et al. Structural Basis for Fluorescence Activation by Pepper RNA. ACS Chem Biol. 2022 Jul 15;17(7):1866-1875.

### Background

HBC599 is a non-fluorescent HBC mimetic that does not emit light in solution but activates and emits bright fluorescence upon forming a complex with the Pepper RNA aptamer. The complex can be detected using an excitation wavelength of 515nm and an emission wavelength of 599nm<sup>[1]</sup>.

### References:

[1] Chen X, Zhang D, Su N, et al. Visualizing RNA dynamics in live cells with bright and stable fluorescent RNAs. Nat Biotechnol. 2019 Nov;37(11):1287-1293.

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