

Data Sheet

# BioTracker™ BSH Activity Bioluminescent Probe

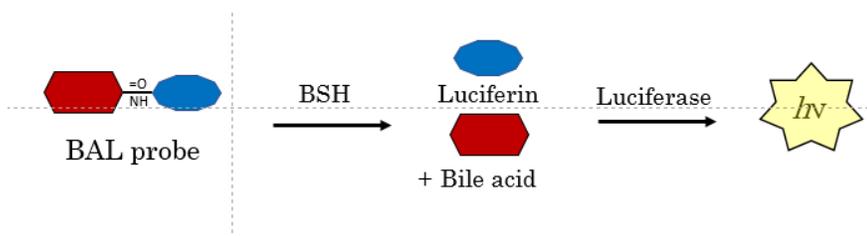
Live Cell Dye

**SCT251****Pack Size: 1 mg****Store at -20 °C****FOR RESEARCH USE ONLY****Not for use in diagnostic procedures. Not for human or animal consumption.**

## Background

The gut microbiome has been increasingly studied for its role in human health. Bile salt hydrolase (BSH), an enzyme produced by gut bacteria, cleaves an amide bond in conjugated bile acids that results in formation of glycine and taurine molecules as well as secondary bile acids. These secondary bile acids are important in host lipid and glucose metabolism as well as gut microbe composition, providing necessary components for gastrointestinal microbe survival. Secondary bile acids also play a role in a variety of health conditions such as colon cancer and age-related diseases relevant to lifespan. Increased BSH activity has positive and diverse benefits such as reduced inflammation, reduced cholesterol levels, improved protection against colon cancer, reduction of urinary tract infections, and even improved symptoms for patients with Crohn's disease. However, the mechanisms by which BSH function is regulated are poorly understood, and knowledge is limited by lack of noninvasive detection methods amenable to use within an intact gut microbiome environment.

The BSH-activatable luciferin (BAL) probe provides a noninvasive optical readout-based method for quantifying BSH activity. This probe is designed to produce bioluminescent light that is proportional to BSH activity. The bile salt hydrolase probe works through a simple mechanism. Structurally, the probe contains a "caged" luciferin molecule that is directly attached to a conjugated bile acid structure. Addition of a BSH-family enzyme will effectively hydrolyze the amide bond which deconjugates the bile acid structure, leaving a free luciferin molecule. This free luciferin can be measured and visualized through a simple luciferase reaction which will result in bioluminescent light production proportional to the amount of probe that is cleaved.



This proportional reaction is eminently useful for assay applications. Biological applications include *in vivo* screening. This assay can also be applied to a variety of samples and alterations. BSH enzyme activity can be measured using pure enzyme, live bacteria, fecal samples, or through the use of *in vivo* noninvasive imaging.

## Source

SCT251 does not contain genetically modified organisms.

## Spectral Properties

This probe is non-fluorescent. It has bioluminescent properties when properly deconjugated.

## Quality Control Testing

Purity:  $\geq 97\%$  confirmed by HPLC, HNMR, LC-MS and elemental analysis. Molar Mass: 653.89 g/mol.

## Storage and Handling

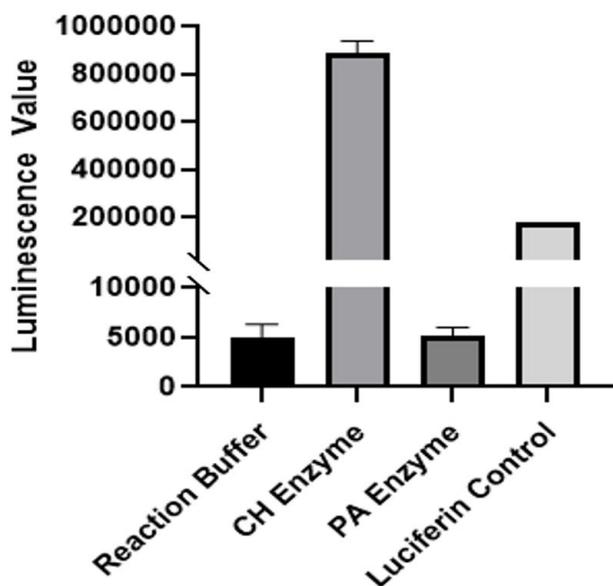
Store Biotracker™ Bile Salt Hydrolase Activity Bioluminescent Probe at -20 °C, desiccated and protected from light.

**NOTE:** Centrifuge vial briefly to collect contents at bottom of vial before opening.

## Presentation

Lyophilized. Yellow solid.

## Representative Data



**Figure 1:** Normalized bioluminescent signals resulting from deconjugation of SCT251 BSH probe (10  $\mu\text{M}$ ) incubated in the reaction buffer containing either choloylglycine hydrolase (CH), penicillin amidase (PA) or free luciferin substrate. Reaction buffer contains 2 mM  $\text{Mg}_2\text{SO}_4$ , 2 mM ATP, 20  $\mu\text{g}/\text{mL}$  Firefly Luciferase enzyme and 10  $\mu\text{M}$  SCT251 bile salt hydrolase probe.

### Reaction Buffer control

Negative control in which the BSH-family enzyme was not added to the reaction buffer.

### Choloylglycine Hydrolase (CH)

5 units/mL CH enzyme (C4018) was added to the reaction buffer and incubated for 1 hour at 37 °C. CH belongs to the BSH family of hydrolases and thus is able to effectively deconjugate the SCT251 probe, releasing the free luciferin to react with luciferase enzyme (SRE0045) and produce a high bioluminescent signal. This illustrates the selectivity of the SCT251 BSH probe for the BSH-family enzymes.

## Penicillin Amidase (PA)

5 units/mL PA enzyme (76427) was added to the reaction buffer and incubated for 1 hour at 37 °C. PA is a non-BSH family enzyme that hydrolyzes similar amide bonds (CO-N) as CH but differs considerably in specificity. The low bioluminescent signal demonstrates the selectivity of the SCT251 BSH probe for BSH-family enzymes and not to the closely related bacterial hydrolase. The SCT251 probe remains conjugated and the luciferin still caged and unable to react to the luciferase enzyme.

## D-Luciferin substrate control

Positive control in which 1 mM D-luciferin substrate (L9504-5MG) was added to the reaction buffer.

## Protocols

### Preparing BioTracker™ Bile Salt Hydrolase Probe stock solution

1. Before opening the vial, briefly centrifuge the vial to collect the solid.
2. Warm the vial to ambient temperature. bile salt hydrolase probe (Molecular Weight: 653.89 g/mol) stock solution by dissolving the contents of one vial (1 mg) in 155 µL of DMSO to create a 10 mM solution.
3. Aliquot and store stock solution stored at -20 °C or below.

### Using Probe

1. Dilute the Bile Salt Hydrolase Probe (SCT251) stock solution (10 mM) 1:1000 in PBS buffer.
2. Prepare Reaction Buffer: 2 mM Mg<sub>2</sub>SO<sub>4</sub>, 2 mM ATP, 20 µg/mL Firefly Luciferase enzyme.
3. Add 10 µM SCT251 probe to the reaction buffer.
4. Add reagents necessary for probe deconjugation (BSH enzymes or other testing reagents)  
Total volume = 100 µL.
5. Add 100 µL of reaction to the necessary wells of a white 96-well flat-bottom plate.
6. Incubate for 60 minutes at 37 °C, protected from light for full probe deconjugation.
7. Obtain results using a plate reader capable of measuring bioluminescence values.

**NOTE:** Optimal concentration must be determined by end user.

## References

1. Sci Adv. 2021, 7(6): eaaz9857.

---

## Notice

We provide information and advice to our customers on application technologies and regulatory matters to the best of our knowledge and ability, but without obligation or liability. Existing laws and regulations are to be observed in all cases by our customers. This also applies in respect to any rights of third parties. Our information and advice do not relieve our customers of their own responsibility for checking the suitability of our products for the envisaged purpose.

The information in this document is subject to change without notice and should not be construed as a commitment by the manufacturing or selling entity, or an affiliate. We assume no responsibility for any errors that may appear in this document.

### Technical Assistance

Visit the tech service page at [SigmaAldrich.com/techservice](https://SigmaAldrich.com/techservice).

### Terms and Conditions of Sale

Warranty, use restrictions, and other conditions of sale may be found at [SigmaAldrich.com/terms](https://SigmaAldrich.com/terms).

### Contact Information

For the location of the office nearest you, go to [SigmaAldrich.com/offices](https://SigmaAldrich.com/offices).

The life science business of Merck KGaA, Darmstadt, Germany operates as MilliporeSigma in the U.S. and Canada.

Merck Biotracker and Sigma-Aldrich are trademarks of Merck KGaA, Darmstadt, Germany or its affiliates. All other trademarks are the property of their respective owners. Detailed information on trademarks is available via publicly accessible resources.

© 2008-2023 Merck KGaA, Darmstadt, Germany and/or its affiliates. All Rights Reserved.

Document Template 20306518 Ver 6.0

00153748 Ver 1.0, Rev 21NOV2023, RC, AB

