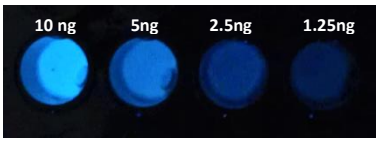
Gaussia princeps Luciferase-Streptavidin Conjugate
Cat. # SA-GLuc

Gaussia Luciferase-Streptavidin conjugates were engineered as followed:

1. Long-lasting mutated Gaussia Luciferase (GLuc-M2) was expressed with a C-terminal AviTag (GLNDIFEAQKIEWHE) in a secretion system to guarantee optimal folding.
2. Subsequently purified GLuc-Avitag protein (>95% purity) was *in vitro* biotinylated using Avidity's biotinylation kit (Cat. # BirA-500)
3. GLuc-Avitag-Biotin was re-purified to remove free biotin and subsequently linked to Streptavidin in a 1:1 ratio. The resulting complex was purified by size exclusion chromatography to remove any free Streptavidin or GLuc.

This product can be used for detection of any biotinylated molecule. Expression of biotinylated membrane proteins can be detected using a luminometer or a light sensitive microscope (e.g. Olympus LV-200).



Dilution series (1:2) of SA-GLuc in a black 96-well plate. Picture was taken with a regular digital camera.

Source: recombinant *Gaussia princeps* M2 luciferase and Streptavidin

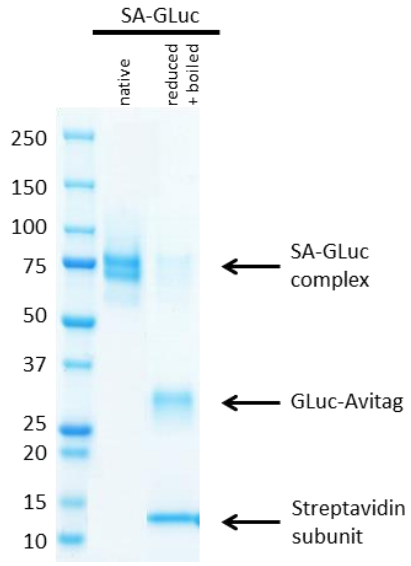
Storage buffer: proprietary, available at www.avidity.com

Storage: The protein is shipped on dry ice and should be immediately stored at -80°C. Protein can be quickly thawed and placed at 4°C. Avoid repeated freeze / thaw cycles.

Concentration: 1 mg/ml determined by Bradford Assay

Purity: >95% by Coomassie staining, affinity and SEC purified

Activity:
- around 3-fold brighter than wildtyp SA-GLuc
- binding efficiency greater than 90%
- very sensitive: use 0.1 to 1 ng of GLuc-SA for biotin coated 96-well plates as control



SDS PAGE of SA-GLuc in native and reduced and oxidized form. One SA-GLuc molecule consists of one GLuc-Avitag protein (27 kDa) and 4 Streptavidin subunits (13 kDa).

Note: Each luminometer has unique thresholds and operations. The user must adapt these guidelines for proper use in the user's instrument. For luminometers that accommodate 96-well plates and have injection ports:

Solutions that contain Gaussia princeps luciferase are diluted in Avidity's Gaussia dilution buffer (GDB). Fifty microliters of luciferase dilution are placed in well. Set up luminometer to inject 100 µl of 100 µM Coelenterazine in PBS plus 1mM EDTA. The luminescence is integrated for 10 seconds with a 2 sec. delay after the injection.

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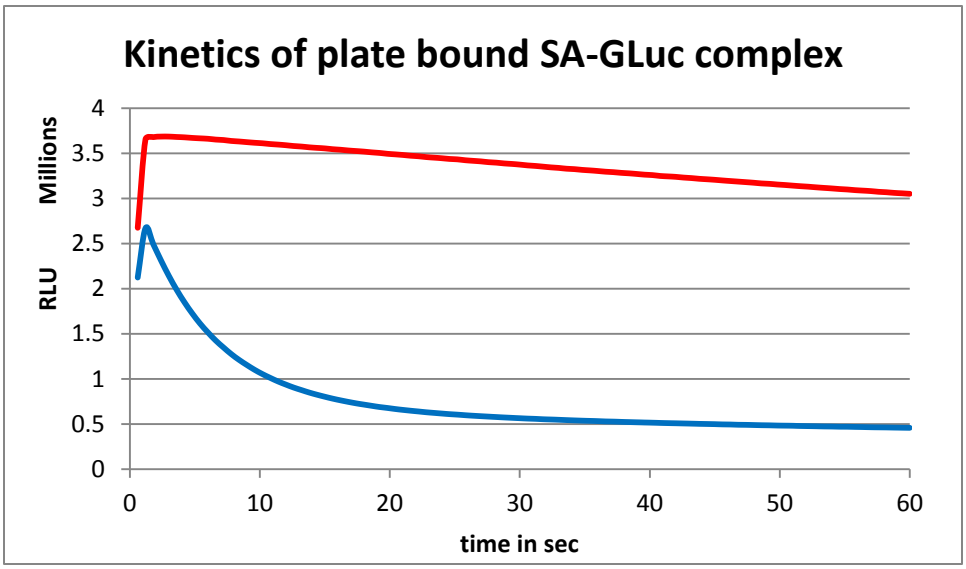
Kinetics of SA-GLuc:

SA-GLuc(M2) and SA-wtGLuc were bound to a 96-well biotin coated plate and washed 5 times with TBS to remove any unbound complexes.

100 µl of 100 µM Coelenterazine was added to each well and the light-output was measured over the next 60 sec.

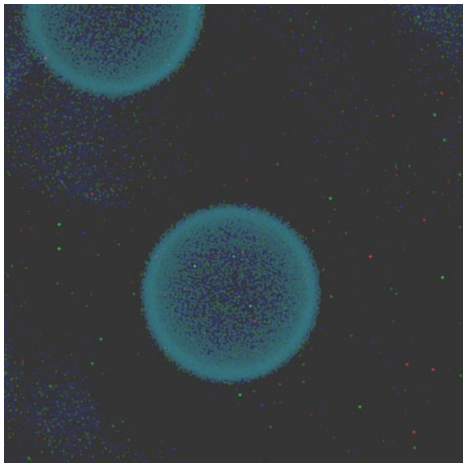


Applications of SA-GLuc:

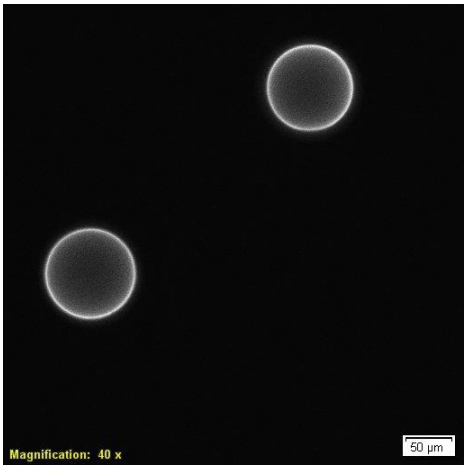
SA-GLuc was bound to Biotin-Agarose (Sigma Cat.# B0519-5ML) at 4°C for 1 hour. Beads were spun down and washed with PBS.

Coelenterazine was added at a final concentration of 100 µM. Agarose beads were examined under a microscope.

The luminescence will last for several minutes and can be increased by adding fresh Coelenterazine substrate.



Biotin-Agarose beads (100 µm) coated with SA-GLuc examined with Nikon microscope with no filter in total darkness.



Biotin-Agarose beads (100 µm) coated with SA-GLuc examined with Olympus LV-200 bioluminescence imaging system.