12. Protocol for SEAP assay in vitro

·Material

Chemiluminescence-based SEAP assay kit (e.g., Sigma Aldrich; cat. no. 1177984200)

buffer (2x): 20 mM L-Homoarginine hydrochloride, 1 mM MgCl2, 21% (v/v) diethanolamine, pH 9.8.

Substrate for SEAP: 120 mM para-nitrophenyl phosphate (pNPP) in 2SEAP buffer.

Transparent 96-well plates.

Colorimetric plate-reader.

·Steps

① Sample 200 μL culture medium from each well, heat inactivate at 65 C for 30 min.

② During the heat inactivation procedure, warm up 2 SEAP buffer (100 μL/well) at 37 ℃.

③ Add 1/5 buffer volume of pNPP (20 μL/well) substrate into the 2x buffer to prepare the “Detection Mixture.”

④ Add 80 μL heated medium into the 96-well plate, add 120 μL Detection Mixture.

⑤ Measure absorption at 405 nm, 30 s per read for 10 reads.

⑥ Calculate enzymatic activity.

·Note

SEAP levels in culture supernatants can be quantified with a pnitrophenylphosphate-based colorimetric assay