

PLCglow

Quality control

Batch number: 201440220

Tested on: July 3rd, 2017

6x assay buffer:

300 mM HEPES (pH 7)

420 mM KCl

18 mM EGTA

12 mM DTT

300 µg/ml bovine serum albumin (fatty acid free)

6x assay buffer with ~10 µM free Ca²⁺:

Same as above plus 17.8 mM CaCl₂

Stock reagents:

10 µM PLCglow: Prepared in 2x assay buffer (+/- free Ca²⁺).

1 nM PLCγ1: Prepared in 1x assay buffer without free Ca²⁺ and serially diluted from purified stock.

Assay:

37.5 µl of 10 µM PLCglow mixed with 30 µl of ddH₂O in a small volume cuvette prior to monitoring fluorescence ($\lambda_{ex/em}$ = 344/535 nm; slits = 3 nm) in a Fluorolog-3 with cuvettes thermostat at 25°C. At time indicated by arrow (**Fig. 1**), 7.5 µl of 1 nM PLC-γ1 added to each condition.

Final assay conditions: 0.1 nM PLC-γ1, 5 µM PLCglow, 50 mM HEPES (pH 7), 70 mM KCl, 3 mM EGTA, 2 mM DTT, 50 µg/ml bovine serum albumin (fatty acid free) and +/- ~10 µM free Ca²⁺.

