Protocol: Hexosaminidase Assay for estimating cell number


Substrate Solution:

Dissolve substrate (p-nitrophenol-N-acetyl-beta-D-glucosaminide, Sigma N-9376, 7.5 mM) and sodium citrate (0.1 M), pH to 5.0. Mix with an equal volume of 0.5% Triton X-100 in water. Aliquot and store at -20.

Per 100 ml:
0.128 g hexosaminidase substrate
1.47 g sodium citrate
250 uL Triton X-100

Developer Solution:

50 mM Glycine
pH 10.4
5 mM EDTA
Aliquot, store at -20

Per 200 mL:
0.75 g glycine
2 mL 0.5 M EDTA

Protocol for 96 well plate:

Remove media from cells. Add 60 ul substrate solution to each well and incubate for a “suitable interval” at 37 C with 100% humidity. To optimize incubation time, make extra wells/plates that you develop early. Your top binding should produce A410 near the top of the readable range. Develop wells by adding 90 ul developer solution – the wells with high cell content should immediately turn visibly yellow. Record absorbance at 410 nm for each well.