Protocol: Hexosaminidase Assay for estimating cell number

This protocol was originally published in Landegren, U. “Measurement of Cell Numbers by Means of the Endogenous Enzyme Hexosaminidase. Applications to Detection of Lymphokines and Cell Surface Antigens” J. Imm. Meth. V. 67 pp 379-388 (1984)

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.