TECHNICAL NOTE

Preparing the Calf Thymus Standard Curve when Using the CytoProbe Hoechst-DNA Assay Kit

Customers have had questions regarding the procedure for preparing the calf thymus standard curve. This technical note is designed to clarify the procedure. Substitute this procedure for that in section 4.2 of the CytoProbe Hoechst-DNA assay kit instructions.

4.2 Prepare the Calf Thymus Standard Curve

To prepare the calf thymus standard curve, follow the steps below.

1. In an Eppendorf tube, combine 368 µl of the 1X TNE with 32 µl of the calf thymus standard to produce a standard concentration of 80 µg/ml (i.e. a 1:12.5 dilution). Mix well by vortexing.

2. Using an eight-channel pipet, add 100 µl of the 1X TNE to the wells that will contain triplicates of the DNA standard curve and the blanks (i.e. rows A-H, columns 1-3)

3. Add 100 µl of the 80 µg/ml DNA standard into each of the first three wells of the 96-well plate (i.e. A1, A2 and A3).

4. Mix the DNA and the TNE in wells A1, A2 and A3 with the eight-channel pipet.

5. Prepare a 1:2 dilution of wells A1, A2 and A3 by adding 200 µl of 1X TNE to the wells and mixing with the eight-channel pipet. These wells now contain the DNA standard at a concentration of 20 µg/ml.

6. Do 1:2 serial dilutions down the plate using 100 µl/well, ending at row G. Mix well at each dilution with the eight-channel pipet. Discard the remaining 100 µl/well of the last dilution, so that all rows from B to G contain the same volume (i.e.100 µl).

***NOTE**: Wells A1, A2 and A3 will have more volume (i.e. 300 µl) than the other wells and should, therefore, be excluded from the calculation of the standard curve.*

Wells	Concentration (µg/ml)
B1, B2, B3	10.0
C1, C2, C3	5.0
D1, D2, D3	2.5
E1, E2, E3	1.3
F1, F2, F3	0.61
G1, G2, G3	0.31
H1, H2, H3	0.00