

## 4– Methyl Umbelliferone Assay Linearity, Reproducibility and Sensitivity

### Introduction

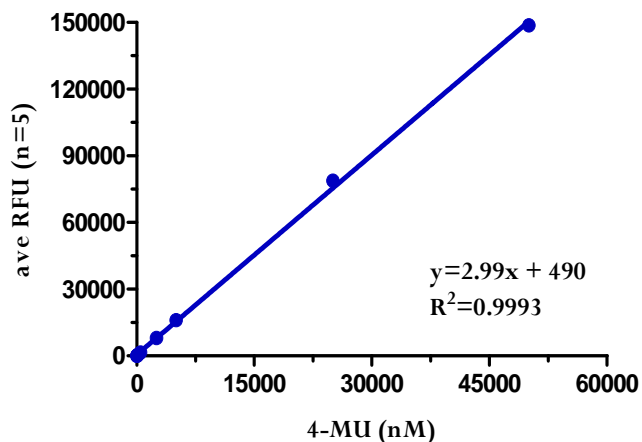
Detection of 4-methyl umbelliferone may be used as a sensitive, quantitative assay for b–galactosidase or other enzymes that cleave substrates linked to 4-MU. Cleavage of 4-methyl umbelliferyl-b-D-galactoside by b-galactosidase yields the fluorescent molecule 4-methyl umbelliferone (7-hydroxy-4 methylcoumarin, 4-MU). The Thermo Scientific NanoDrop™ 3300 Fluorospectrometer can measure as little as 1 ul of sample, thereby significantly scaling-down the reaction volumes commonly needed for conventional cuvette-based fluorometers.

### Method

Serially diluted 4-methyl umbelliferone was measured using the UV LED excitation source with the emission wavelength monitored at 460 nm.

### Results

4-Methyl Umbelliferone Linearity  
NanoDrop 3300



Using a 2ul volume, sensitivity is ~2 pg or 1 pg/ul.

Picograms of 4-MU per assay volume	4-MU nM	Ave RFU (n=5)	Stdev	%CV
2	5	17	0.8	4.7
10	25	78	0.5	0.6
20	50	154	1.3	0.8
100	250	799	7.3	0.9
200	500	1602	8.1	0.5
1000	2500	8089	115.0	1.4
2000	5000	16039	100.0	0.6
10000	25000	78829	603.0	0.8
20000	50000	148668	1307.0	0.9