EROD Assay

1. Prepare buffers.

TEDG:

Tris-HCL	50 mM, pH 7.4
EDTA	1 mM
DTT	1 mM
Glycerol	20%

Assay Buffer:

Tris	50 mM, pH 7.8
NaCl	0.1 M

NADPH (1.67 mM = final concentration in assay):
Dilute NADPH (Sigma, N-1630) at a concentration of 5.57 mg/ml is Assay Buffer.

7-Ethoxyresorufin:
Prepare a saturated solution in methanol by adding 1 ml of MeOH to 1 mg of 7-ER.
This stock can be stored at -20°C in an amber vial.
Warm the 7-ER solution with hot tap water, vortex and let settle. This saturated solution is ~400uM.

Prepare 2.67 uM 7-ER for use in assays in 15 or 50 ml conical tubes by adding 66 ul of the saturated solution per 10 mls of assay buffer. To determine if the concentration is correct, the A482 should be 0.060 (Ext. coeff. = 22.5 mM-1cm-1). Add more 7-ER or assay buffer to reach this absorbance.
This solution should be wrapped in foil and may be stored at -20°C and reused for up to 1 week.

2. Prepare standard curve. It is sufficient to run a standard curve once every few months.

Prepare a stock methanol solution of resorufin by dissolving a few crystals in methanol. Take 100 ul of the concentrated methanol solution and dilute it in 10 ml of EROD assay buffer. Determine the concentration by reading the absorbance at 574nm. Divide the absorbance by the ext. coeff (45.78 mM-1cm-1). Then prepare 10 ml of 2 μM resorufin in assay buffer.
Using the 2 μM resorufin, dilute with assay buffer to prepare a series of standards ranging from 5 – 200 pmol in 100 μl.

Standard	Final Volume of Standard	Vol of 2 uM Stock	Vol of Assay Buffer
pmol	ul	ul	ul
0	1000	0	1000
5	1000	25	975
10	1000	50	950
20	1000	100	900
40	1000	200	800
80	1000	400	600
100	1000	500	500
150	1000	750	250
200	1000	1000	0

Add 100 ul of each standard to duplicate wells.
Read on a fluorometric plate reader with an excitation filter of 530/25 nm and an emission filter of 620/40 nm.
If planning on using different gain settings to run your samples, be sure to analyze the standard curve at each gain setting.

3. Read samples
Thaw microsomes on ice.
Thaw NADPH on ice, then warm to RT right before using.
Warm 7-ER to RT.

Pipette 5 μl of each sample in duplicate wells in a black-sided, clear bottomed 96-well plate. Samples expected to have high EROD activities should be diluted 1:10.
Using a repeater pipetter, add 80 μl of the 7-ER in assay buffer.
Right before reading on the fluorometer, add 20 μl of the NADPH.

The fluorometer should be set at 37°C for mammalian samples.
Read with an excitation of 530/25 nm and an emission of 620/40 nm.
Samples should be read at 1-5 min intervals for 10-30 min, depending upon the expected activity.

RFUs/min should be determined from a linear curve fit of the data and converted to pmol/min/mg using the standard curve and sample protein concentration.