Dopamine D47 (RBHD47M400UA) saturation curve

Assay buffer:	50 mM Tris-HCl pH 7.4, 120 mM NaCl, 5 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA		
Wash buffer:	50 mM Tris-HCl pH 7.4, 0.9 % NaCl		
Radioligand	[3H]-MethylSpiperone	85.5 Ci/mmol	1 µCi/1 µl
Cold ligand	Haloperidol		
Unit size	35 µg protein / unit		
Protein concentration	14 mg/ml		

	Full scale assay format
Total volume	**550 µL**
Volume membranes	500
Volume cold ligand or buffer	25
Volume radioligand	25

Membrane dilution

0.125ml membranes + 24.875 ml assay buffer (1:200 dilution)

Haloperidol dilution

Stock concentration	10 mM = 10 000 µM
Prepare 1000µL in assay buffer	
Final concentration	25 µM
Dilution factor	22
Intermediate concentration	550 µM

Volume haloperidol stock = $\frac{1000\ \mu L * 550\ \mu M}{10\ 000\ \mu M}$ =55 µL stock

Radioligand dilution

Need 600 µL tube 1

Final Concentration 5 nM

Intermediate concentration 110 nM

110 nmol / 1000 ml	x	85.5 uCi / nmol	x	1ul / 1uCi	x	0.6 ml	=	5.6 ul

*Correction factor (1.3x) 5.6µL x 1.3 = **7.3µL**

Tube #	Final Concentration (nM)	Volume stock	Volume assay buffer
1	5	7.3 µl	593 µL
2	2	200 µl tube 1	300 µL
3	1	100 µL tube 1	400 µL
4	0.5	50 µL tube 1	450 µL
5	0.2	50 µL tube 2	450 µL
6	0.1	50 µL tube 3	450 µL
7	0.05	50 µL tube 4	450 µL
8	0.02	50 µL tube 5	450 µL

*Correction factor is used to account for ligand sticking to plastic; actual ligand concentration is later determined by spotting a set volume of working material from each tube onto a Filtermat, measuring dpm, and converting to moles using specific activity.

Plate map

Radioligand Concentration (nM)	Full Scale Saturation (550ul)					
	Total			Non-Specific		
	1	2	3	4	5	6
5	1	1	1	1	1	1
2	2	2	2	2	2	2
3	3	3	3	3	3	3
4	4	4	4	4	4	4
5	5	5	5	5	5	5
6	6	6	6	6	6	6
7	7	7	7	7	7	7
8	8	8	8	8	8	8

1) In DeepWell plate:
   a. Add 25 µL buffer or Haloperidol
   b. Add 25 µL radioligand
   c. Add cell membranes (500 uL)
2) Incubate 1 hour
3) During incubation, pre-soak a UniFilter plate in 0.01% PEI
4) Wash assay plate over cell harvester, onto UniFilter plate
5) Dry UniFilter plate in 27°C oven
6) Add BackSeal to bottom of UniFilter plate
7) Add scintillation cocktail and TopSeal
8) Measure