PDSP - Protocol

5-HT Receptor Subtypes

- 5-HT Receptor
- DA Receptor
- MUSCARINIC
- ADRENERGIC
- OPIATE Receptors
- NICOTINIC Receptors
- GABA Receptors
- IONOTROPIC GLUTAMATE
- PEPTIDE Receptors
- TRANSPORTER BINDING

For each receptor class, one binding assay is described in detail. All binding assays are carried out with an incubation time of 60 MIN/RT (RT=room temp). All other binding assays are performed in a similar manner, although the buffers used differ from assay to assay. The table below gives the (1) radioligand; (2) assay buffer; (3) unlabelled reference ligand; and (4) references to demontrate expertise.

Serotonin	Radioligand	Assay buffer	Unlabelled ligand as reference compound	References
5-HT1A	3H-8-OH-DPAT	A	WAY 100635	Roth lab standard protocol
5-HT1B	125I-IODO-CYAN-OPINDOLOL	B	CGS-12066A	Roth lab standard protocol
5-HT1Da	3H-5-CARBOXIMIDO-TRYP-TAMINE (5-CT)	B	Metergoline	Roth lab standard protocol
5-HT1Db	3H-5-CT	B	Metergoline	Roth lab standard protocol
5-HT1E	3H-5-HT	C	Methiothepine	Roth lab standard protocol
5-HT1F	3H-5-HT	C	Methiothepine	Roth lab standard protocol
5-HT2A	3H-ketanserin	A	Spiperone	
5-HT2B	3H-mesulergine	A	LY53857	
5-HT2C	3H-mesulergine	A	LY53857	
5-HT4	3H-GR113808	A	SDZ 205,557	Roth lab standard protocol
5-HT3	3H-Chlorophenylbiguanide	D	ICS 205,930	
5-HT5a	3H-LSD	D	Ergotamine	Roth lab standard protocol
5-HT6	3H-LSD	A	CLOZAPINE	
5-HT7	3H-LSD	A	CLOZAPINE	

Assay buffers:

A=50 mM Tris-Cl, 0.5 mM EDTA, 5 mM $MgCl_2$, pH=7.4
B=50 mM Tris-Cl, 154 mM NaCl, 0.5 mM EDTA, 5 mM $MgCl_2$, pH=7.4

C=50 mM Tris-Cl, 0.5 mM EDTA, 5 mM $MgCl_2$, 0.01% ascorbic acid, 10 uM pargylline; pH=7.4
D=50 mM Tris-Cl, pH=7.4
E=20 mM Hepes, 5 mM MgCl2, 10 ug/ml aprotinin, 1mg/ml bacitracin, 0.3% bovine serum albumin (BSA).
F=Phosphate buffered saline, 10 mM MgCl2, 2 mM EGTA, 0.15 mM bacitracin, 1.5% BSA, pH=7.0
G=50 mM Tris-Cl, 150 mM NaCl, 5 mM KCl, pH=7.40

Assay description for 5-HT receptors:

Set up 96-well plate as follows:

UNK #1	UNK #1	UNK #2	UNK #2	UNK #3	UNK #3	UNK #4	UNK #4
UNK #5	UNK #5	UNK #6	UNK #6	UNK #7	UNK #7	UNK #8	UNK #8
UNK #9	UNK #9	UNK #10	UNK #10	UNK #11	UNK #11	UNK #12	UNK #12
STANDARD #1	STANDARD #1	STANDARD #2	STANDARD #2	TOTAL BINDING	TOTAL BINDING	TOTAL BINDING	TOTAL BINDING
STANDARD #1 AT 1 nM	STANDARD #1 AT 1 nM	STANDARD #1 AT 3 nM	STANDARD #1 AT 3 nM	STANDARD #1 AT 10 nM	STANDARD #1 AT 10 nM	STANDARD #1 AT 30 nM	STANDARD #1 AT 30 nM
STANDARD #1 AT 100 nM	STANDARD #1 AT 100 nM	STANDARD #1 AT 300 nM	STANDARD #1 AT 300 nM	STANDARD #1 AT 1000 nM	STANDARD #1 AT 1000 nM	TOTAL COUNTS ADDED / PLATE	TOTAL COUNTS ADDED / PLATE

1 For 3H-ligands use 1-2 nM final concentration of radioligand; for 125I-radioligands, use 0.05-0.1 nM final concentration.

2 Pipette in following order:

--Binding buffer

--Radioligand

--Cold unknown ligand

--Cold reference ligand

--Membranes

3 Incubate at RT as listed in binding assay table above.

4 At the end of the incubation, harvest onto pre-soaked (0.3% polyethyleneimine) GF/C filters using 96-well harvester. Wash with 3 quick washes with ice-cold harvest buffer.

5 Remove filters and air dry overnight.

6 Count in 96-well counter using 100 ul of scintillant/well.

7 Calculate Ki value of reference compound using LIGAND Program.

8 Calculate % inhibition using following formula:

$$\% \text{ Inhibition} = \frac{\text{Counts bound at 10 uM concentration of unknown compound}}{\text{Total specific counts}} \times 100$$

9 Down-load all raw data to Dr. Friedman for quality control studies.