

Product Information

D₃ Dopamine Receptor from rat, recombinant expressed in Sf9 cells

Product Code **D-181**
Storage Temperature $-70\text{ }^{\circ}\text{C}$

Product Description

Dopamine receptors were initially divided into two general categories on the basis of differences in receptor pharmacology and biochemical mechanisms of signal transduction. With the application of molecular biology techniques, two predominant dopamine receptors, D₁ and D₂, were cloned. Later other dopamine receptors with homology to either the D₁ or D₂ receptor were identified. Thus, at present, two families of vertebrate dopamine receptors (designated as D₁-like and D₂-like) are recognized. The D₁-like family consists of the D₁ and D₅ receptors, while the D₂-like family consists of the D₂, D₃, and D₄ receptors.

The D₁ and D₂ receptors occur in sufficiently high concentrations that they can be studied *in situ*. The D₃, D₄, and D₅ receptors occur at such low concentrations that study of them *in situ* is difficult. Thus, the majority of the research on these receptors has been accomplished using cell lines cloned to express these receptors.

Reagents

This product is provided as purified membranes suspended in 50 mM Tris-HCl, pH 7.4, containing 10% glycerol and 1% bovine serum albumin (BSA).

Storage/Stability

The product ships on dry ice and it is recommended to store tightly sealed at $-70\text{ }^{\circ}\text{C}$. When stored in the supplied solution, the membranes retain their original specific activity for several months.

Procedure

1. Prepare Diluted Membranes in incubation buffer (0.5 ml of membranes to 24.5 ml of incubation buffer). Incubation buffer contains 50 mM Tris-HCl, pH 7.4, with 5 mM MgCl₂, 5 mM EDTA, 5 mM KCl, 1.5 mM CaCl₂, and 120 mM NaCl.

2. Assay mixture:
500 μl of Diluted Membranes
20 μl of [³H]-Radioligand in buffer
20 μl of incubation buffer or unlabeled ligand in buffer

Radioligand: [³H]-Spiperone at a final concentration of 0.4 nM for competition studies.
Unlabeled ligand: Haloperidol at a final concentration of 10 μM .

3. Incubate for 60 minutes at 27 $^{\circ}\text{C}$.
4. Separation:
Over GF/C filter (5 mm diameter, presoaked in 0.3% polyethylenimine) then wash 9 times with 500 μl of ice cold 50 mM Tris-HCl, pH 7.4.

Results

Typical affinities using standard binding assay above. Results may vary from lot to lot.

Ligand	Affinity (K _D) (nM)
[³ H]-Spiperone	0.62
Chlorpromazine	6.7
Haloperidol	17
(-)-Sulpiride (S-112)	26
SKF 38393 (D-047)	3788

References

1. Swarzenski, B.C., et al. Morphogenic potentials of D₂, D₃, D₄, dopamine receptors revealed in transected neuronal cell lines. Proc. Natl. Acad. Sci. USA, **91**, 649-653 (1994).

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