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ProductInformation

D_1 DOPAMINE RECEPTOR, HUMAN
Expressed in SF9 Cells

Product Number **D-178**

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 the techniques of molecular biology, two predominant dopamine receptors, D_1 and D_2, were cloned. Later other dopamine receptors with homology to either the D_1 or D_2 receptor were identified. Thus, at present, two families of vertebrate dopamine receptors (designated as D_1-like and D_2-like) are recognized. The D_1-like family consists of the D_1 and D_5 receptors while the D_2-like family consists of the D_2, D_3 and D_4 receptors.

The D_1 and D_2 receptors occur in sufficiently high concentrations that they can be studied *in situ*. The D_3, D_4 and D_5 receptors occur in such low concentrations that study of them *in situ* is difficult. Thus, the majority of study of these receptors has been accomplished using cell lines cloned to express these receptors.

Reagents

D_1 Dopamine Receptor, Human (Sf9) is provided as purified membranes suspended in 50 mM Tris-HCl at pH 7.4, containing 10% glycerol and 1% bovine serum albumin (BSA).

Storage/Stability

Store tightly sealed at –80 °C. When stored in its original packaging solution, the membranes retain their original specific activity for several months.

Procedure

Incubation Buffer
50 mM Tris-HCl at pH 7.4, containing 5.0 mM $MgCl_2$, 5.0 mM EDTA, 5.0 mM KCl and 1.5 mM $CaCl_2$
Typical Binding Protocol

1. Membranes
   Dilute in incubation buffer (0.5 ml of membranes to 24.5 ml of incubation buffer).
2. Assay mixture
   500 µl of diluted membranes
   20 µl of [^{3}H]-radioligand in buffer
   20 µl of incubation buffer or unlabeled ligand in buffer
   Radioligand: [^{3}H]-SCH-23390 at a final concentration of 1.6 nM for competition studies.
   Unlabeled ligand: (+)-Butaclamol (Cat. No. D-033) at a final concentration of 10 µM.
3. Incubation time
   90 minutes at 27 °C.
4. Separation
   Over GF/C filter (5 mm diameter, presoaked in 0.3% polyethylenimine, Cat. No. P-182) then wash 4x with 500 µl of ice cold 50 mM Tris-HCl at pH 7.4.

Results

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

Ligand	Affinity (K_i) (nM)
[^{3}H]-SCH-23390	1.3 (K_d)
(+)-Butaclamol (D-033)	1.6
SKF 83566 (S-110)	3.6
Haloperidol (H-100)	38
Spiperone (D-050)	261

References

1. Jarvie, K.R., et al., "Molecular cloning, stable expression and desensitization of the human dopamine D_{1b}/D_5 receptor." *J. Recept. Res*. **13**, 573-590 (1993).
2. Sunahara, R.K., et al., "Cloning of the gene for a human D_5 receptor with higher affinity for dopamine than D_1." *Nature* **350**, 614-619 (1991).
3. MacKenzie, R.G., et al., "A D_1/D_2 chimeric dopamine receptor mediates a D1 response to a D_2-selective agonist." *FEBS Lett*. **323**, 59-62 (1993).

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