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ProductInformation

GABAERGIC LIGAND-SET™

Product Number **L7884**
Storage Temperature -20°C

Product Description

The GABAergic LIGAND-SET™ is a set of 40 small organic ligands that modulate GABA receptors. These ligands are arrayed in a standard 96-well plate format; , each well has a capacity of 1 ml.

This set can be used for screening new drug targets, for guiding secondary screens of larger, more diverse libraries and for standardizing and validating new screening assays.

There are three classes of γ-aminobutyric acid (GABA) receptors, $GABA_A$, $GABA_B$ and $GABA_C$. $GABA_A$ and $GABA_C$ are ligand gated ion channels, while $GABA_B$ is a G protein-coupled receptor. There are two isoforms of $GABA_B$ receptors: GBR1a and GBR1b (molecular weight of 130 kDa and 92 kDa, respectively).

$GABA_A$ receptor activation induces a cascade of events that culminates in the influx of Cl^- into neurons and the hyperpolarization of postsynaptic neuronal membranes, with a consequent reduction in neuronal excitability. $GABA_A$ receptors are composed of five subunits derived from four subunit families (α, β, γ and δ). The potency and efficacy of GABA, benzodiazepines (BZDs) and non-BZD ligands to activate or modulate GABA action at $GABA_A$ receptors is influenced by the assembly of subunits that form the receptor. Thus GABA binds to α and β subunits whereas BZDs bind only to α and γ subunits.

Recent studies using the high affinity radiolabeled ligand, [^{125}I]-CGP 64213, have demonstrated that the active $GABA_B$ receptor is a heterodimer.. In early studies of the $GABA_B$ receptor agonists did not activate recombinant receptors expressed in cell lines as they did in homogenates of cells containing the natural receptor. It was later found that the natural $GABA_B$ receptor was actually a heterodimer, with a second 'receptor', designated GBR2, linked to the GBR1 at the C-terminus. . GBR2 is structurally similar to GBR1, in that is has a high molecular weight (110 kDa), seven transmembrane-spanning domains, and a long extracellular chain at the N-terminus. However it is unclear as to whether it is really a receptor or a trafficking protein, as no binding site for GABA or any modulators have been detected on GBR2. It is possible that the two function together to amplify GABAegic signaling.

$GABA_C$ receptors were first proposed in 1986 to describe a bicuculline- and baclofen-insensitive [^{3}H]-GABA binding site on cerebellar membranes. They have subsequently been shown to be ligand-gated chloride channels. These receptors are highly sensitive to GABA, but are not blocked by traditional $GABA_A$ receptor antagonists. Furthermore, they are not modulated by $GABA_A$ receptor modulators. Finally, $GABA_C$ receptors are insensitive to baclofen, a highly selective $GABA_B$ receptor agonist, and to phaclofen and saclofen, two $GABA_B$ receptor antagonists. Most of the knowledge of $GABA_C$ receptors comes from studies of the visual system, however evidence does exist that they are present in other brain regions.

Components/Reagents

The GABAergic LIGAND-SET™ contains 2 mg of each ligand per well. Stock solutions can be readily prepared by adding 1 ml of DMSO to each well. The set also comes with a diskette containing a structure database, or SD file, and a Microsoft Excel file containing the catalog number, name, rack position and pharmacological characteristics of each ligand. The following is a listing of all the ligands included:

21,167-2	(±)-Nipecotic acid
A-120	5-Aminovaleric acid hydrochloride
A-196	3-Aminopropyl-(methyl)phosphinic acid hydrochloride
A-201	cis-4-Aminocrotonic acid
A2129	γ-Amino-n-butyric acid (GABA)
A4147	4-Amino-1-propanesulfonic acid
A6566	2-Hydroxysaclofen
A7162	3-Aminopropylphosphonic acid

B-020	(±)-Baclofen
B-103	(-)-Bicuculline methbromide, 1(S), 9(R)
B9130	(+)-Bicuculline
C0424	PK 11195
G-013	R(+)-Baclofen hydrochloride
D8555	N,N-Dihexyl-2-(4-fluorophenyl)indole-3-acetamide
E-001	Ethyl β-carboline-3-carboxylate
E-002	Methyl β-carboline-3-carboxylate
E-006	N-Methyl-β-carboline-3-carboxamide
E-007	Methyl-6,7-dimethoxy-4-ethyl-β-carboline-3-carboxylate
F6300	Flumazenil
G-002	Isoguvacine hydrochloride
G-007	Guvacine hydrochloride
G-019	Muscimol hydrobromide
H8645	(+)-Hydrastine
I0375	Imidazole-4-acetic acid hydrochloride
M2922	3-Methyl-6-(3-[trifluoromethyl]phenyl)-1,2,4-triazolo[4,3-b]pyridazine
N-142	NO-711 hydrochloride
P-118	Phaclofen
P-162	Pregnenolone sulfate sodium
P1675	Picrotoxin
P7295	Primidone
P8390	Picrotoxinin
P9159	Piperidine-4-sulphonic acid
R-109	Ro 15-4513
R-110	Ro 05-3663
S-106	SR-95531
S-166	Saclofen
T-101	THIP hydrochloride
T-112	Tracazolate
T-200	TPMPA
Z4900	Zopiclone

Preparation Instructions

To create a new database in ISIS™/BASE :

- Open ISIS™/BASE.
- Choose **File>New database**.
- Enter **GABAergic** or a preferred name in the File name field.
- Click **Save**.

The "Create Database" window will now be open.

- Enter **Catnum** for the Field name.
- Choose **Variable text** from the drop down window of the Type field.
- Click Add.
- Repeat the above steps for the following:

Field name	Type
Name	**Variable text**
Position	**Variable text**
Action	**Variable text**
Class	**Variable text**
Selectivity	**Variable text**
SecName	**Variable text**
Description	**Variable text**

- Enter **Structure** for the Field name.
- Choose **Structure** from the drop down window of the Type field.
- Enter ***Structure** for the External name.
- Click **Add**.
- Click **Save**.

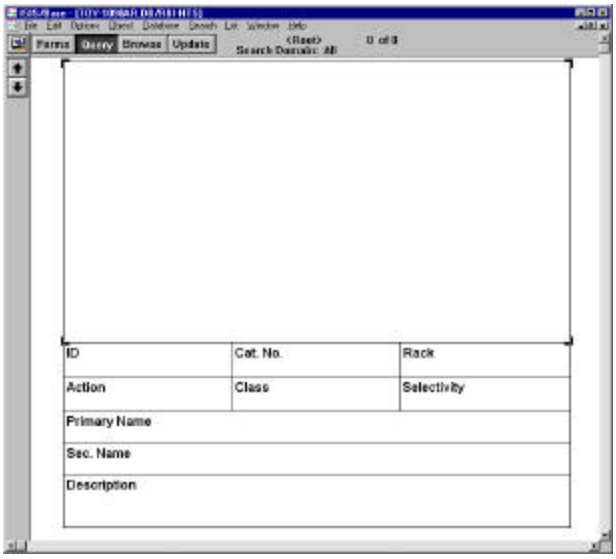
The main ISIS™/BASE window will now be open.

To create the Form:

- Click on the "Draw a box" button (second button down on the left of the screen).
- Move the mouse to the bottom left hand corner and draw a box, ½ inch high, the length of the screen by clicking on the left mouse button and dragging the mouse across the screen. (see figure below)
- Above this box, draw another ½ inch high box the length of the screen. (see figure below)
- Above this box, draw a third ½ inch high box the length of the screen. (see figure below)
- Above these long boxes draw 3 ½ inch high x 3 inch wide boxes. (see figure below)
- Above these 3 boxes, draw another three the same size. (see figure below)
- Draw a final box to fit the remaining space of the screen above these boxes. (see figure below)

Double click on the top box. This will open the Box properties window.

- Click on **Structure**.
- Click **OK**.



- Repeat the same steps, clicking on the appropriate field name for the appropriate box:

Box	Field name
First small box	**ID**
Second small box	**Catnum**
Third small box	**Position**
Fourth small box	**Action**
Fifth small box	**Class**
Sixth small box	**Selectivity**
First long box	**Name**
Second long box	**SecName**
Bottom long box	**Description**

- Choose **File>Save form**.
- Enter GABAergics or preferred name.
- Click **OK**.

Importing an SD file:

- Click **Update**.
- Choose **File>Import>SD File**. ***NOTE:*** *For MAC users, you must hold down the* ***option key*** *while choosing* **File>Import>SD File**. *If you do not, the GABAergic.sdf will not be visible in the import window.*
- Enter **GABAergic.sdf** (Located on the floppy diskette provided with the plate).
- Click **Open**.

  The Import SD File window will now be open.
- Click on **Add a new record including structure**, on both sides of the table.
- Click **OK**.

The database is now ready to use.

Storage/Stability

Store plate -20°C with cap strips firmly in place. Plate cover should only be removed when plate is in use to prevent loss of caps strips.

References

1. Chebib, M., et al., "Analogues of γ-aminobutyric acid (GABA) and trans-4-aminocrotonic acid (TACA) substitute in the 2 position as $GABA_C$ receptor antagonists." *Br. J. Pharmacol.*, **122**, 1551-1560 (1997).
2. Bowery, N., "Metabotropic $GABA_B$ receptors." *Neurotransmissions*, **15(2)**, 3-11 (1999).
3. Costa, E. and Guidotti, A., "Benzodiazepines on trial: A research strategy for their rehabilitation." *Trends Pharmacol. Sci.*, **17**, 192-200 (1996).

SMS 8/00