eurofins | Discovery Services

PRODUCT DATASHEET

Ready-to-Assay™ $GABA_B$ GABA Family Receptor Frozen Cells

CATALOG NUMBER: HTS119RTA

CONTENTS: Pack contains 2 vials of mycoplasma-free cells, 1 ml per vial. Fifty (50) mL of Media Component.

STORAGE: Vials are to be stored in liquid N_2. Media Component at 4ºC (-20ºC for prolonged storage).

BACKGROUND

Ready-to-Assay GPCR frozen cells are designed for simple, rapid calcium assays with no requirement for intensive cell culturing. Eurofins Discovery Services has optimized the freezing conditions to provide cells with high viability and functionality post-thaw. The user simply thaws the cells and resuspends them in media, dispenses cell suspension into assay plates and, following over night recovery, assays for calcium response.

The neurotransmitter γ-aminobutyric acid (GABA) exerts its effects through an ion channel, $GABA_A$, and a GPCR, $GABA_B$. Functional $GABA_B$ is a heterodimer composed of the $GABA_{B1}$ and $GABA_{B2}$ subunits, which share 35% sequence identity and belong to the class 3 family of GPCRs. The $GABA_{B1}$ subunit, which exists as splice variants $GABA_{B1a}$ and $GABA_{B1b}$, binds directly to GABA and is required for agonist activation. The $GABA_{B2}$ and $GABA_{B1}$ subunits associate by formation of a coiled coil by their C-terminal tails; this association masks an ER retention sequence in $GABA_{B1}$ to permit export from the ER and trafficking to the cell surface. In addition to its chaperone function, $GABA_{B2}$ is the component that couples to G_i to reduce intracellular cAMP. Agonists of $GABA_B$, such as baclofen, are used clinically for treatment of muscle spasticity, migraine headache and musculoskeletal pain (Bowery *et al.*, 2002). Cloned human $GABA_B$-expressing cell line is made in the Chem-1 host, which supports high levels of recombinant $GABA_B$ expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. Thus, the cell line is an ideal tool for screening for agonists, antagonists and modulators at $GABA_B$.

USE RESTRICTIONS

Please see User Agreement (Label License) for further details. ***One such restriction is that the contents of the supplied vial(s) are limited to a single use and shall not be propagated and/or re-frozen by licensee.***

WARNINGS

For Research Use Only; Not for Use in Diagnostic Procedures
Not for Animal or Human Consumption

GMO

This product contains genetically modified organisms.
Este producto contiene organismos genéticamente modificados.
Questo prodotto contiene degli organismi geneticamente modificati.
Dieses Produkt enthält genetisch modifizierte Organismen.
Ce produit contient organismes génétiquement des modifiés.
Dit product bevat genetisch gewijzigde organismen.
Tämä tuote sisältää geneettisesti muutettuja organismeja.
Denna produkt innehåller genetiskt ändrade organismer.

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APPLICATIONS

Calcium Flux Assays

APPLICATION DATA

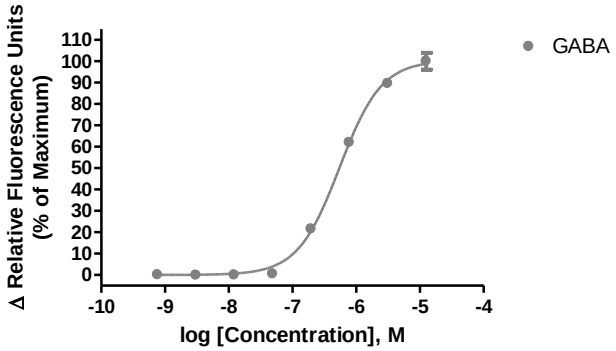

Figure 1. Representative data for activation of $GABA_B$ receptor. Calcium flux in $GABA_B$–expressing Chem-1 cell line induced by GABA. $GABA_B$–expressing Chem-1 cells were loaded with a calcium dye, and calcium flux in response to the indicated ligand(s), 4-fold serial dilution with each concentration performed in duplicate, was determined on a Molecular Devices $FLIPR^{TETRA}$. Maximal fluorescence signal obtained in this experiment was 2,800 RLU (Relative Light Units).

Table 1. EC_{50} value of $GABA_B$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY (nM)	REFERENCE
GABA	Calcium Flux	550	Eurofins Internal Data

ASSAY SETUP

1. Immediately upon receipt, thaw cells or place cells in liquid nitrogen.
2. Thaw cells rapidly by removing from liquid nitrogen and immediately immersing in a 37ºC water bath. Immediately after ice has thawed, sterilize the exterior of the vial with 70% ethanol.
3. Add 1mL of pre-warmed Media Component to each vial of cells. Place contents from two vials into a 15 mL conical tube and bring the volume to 10 mL of Media Component.
4. Centrifuge the cell suspension at 190 x g for four minutes
5. Remove supernatant and add 10.5 mL of pre-warmed Media Component to resuspend the cell pellet.
6. Seed cell suspension into appropriate assay microplate (100 µL/well for 96-well plate, 25 µL/well for 384-well plate).
7. When seeding is complete, place the assay plate at room temperature for 30 minutes.
8. Move assay plate to a humidified 37ºC 5% CO2 incubator for 24 hours.
9. After 24 hour incubation, remove assay plate from the incubator and remove Media Component.
10. Prepare Fluo-8, AM (AAT Bioquest: 21080) Ca^{2+} dye by dissolving 1mg of Fluo-8 NW in 200 µL of DMSO. Once dissolved place 10 µL of Fluo-8 NW Ca^{2+} dye solution into 10 mL of HBSS 20mM HEPES, 2.5mM Probenecid pH 7.4 buffer and apply to assay microplate (Ca^{2+} dye at 10 µL /10 mL is sufficient for loading one (1) microplate).
11. Set-up FLIPR to dispense 3x ligand to appropriate wells in the assay plate. Set excitation wavelength at 470-495 nm ($FLIPR^{TETRA}$) or 485 nm (FLIPR1, FLIPR2, FLIPR3) and emission wavelength at 515-565 nm

(FLIPR^{TETRA}) or emission filter for Ca^{2+} dyes (FLIPR1, FLIPR2, FLIPR3). Set pipet tip height to 5 µL below liquid level and dispense rate to 75 µL/sec (96-well format) or 50 µL/sec (384-well format). Set up plate layout and tip layout for each individual experiment. Set time course for 180 seconds, with ligand addition at 10 seconds.

12. Ligands are prepared in non-binding surface Corning plates (Corning 3605 – 96-well or Corning 3574 – 384-well).
13. After the run is complete, negative control correction is applied and data analyzed utilizing the maximum statistic.

ASSAY MATERIALS

Description	Supplier and Product Number
HBSS	Hyclone: SH3026802
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma: P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
GABA ligand	Tocris: 0344
Non-binding white plates (for ligand prep)	Corning: 3605(96-well)/3574(384-well)
Black (clear bottom) tissue-culture treated plates	Corning: 3904(96-well)/3712(384-well)

FLIPR SETTINGS

Settings for FLIPR^{TETRA}® with ICCD camera option

Option	Setting
Read Mode	Fluorescence
Ex/Em	Ex470_495 / Em515_575
Camera Gain	2000
Gate Open	6 %
Exposure Time	0.53
Read Interval	1s
Dispense Volume	50 µl (25 µl for 384-well)
Dispense Height	25 µl (50 µl for 384-well)
Dispense Speed	75 µl L/sec (50 µl for 384-well)
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

HOST CELL

Chem-1, an adherent rat hematopoietic cell line expressing endogenous Gα15 protein.

EXONGENOUS GENE EXPRESSION

GABBR1 & GABBR2 cDNA (Accession Number: NM_021903 & NM_005458, respectively; see CODING SEQUENCE below) expressed from a proprietary pHS plasmid.

CODING SEQUENCE

GABBR1

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                                    ATG GGG CCC GGG GCC CCT TTT GCC CGG GTG GGG TGG CCA CTG CCG
                                      M   G   P   G   A   P   F   A   R   V   G   W   P   L   P

CTT CTG GTT GTG ATG GCG GCA GGG GTG GCT CCG GTG TGG GCC TCC CAC TCC CCC CAT CTC CCG CGG CCT
 L   L   V   V   M   A   A   G   V   A   P   V   W   A   S   H   S   P   H   L   P   R   P

CAC TCG CGG GTC CCC CCG CAC CCC TCC TCA GAA CGG CGC GCA GTG TAC ATC GGG GCA CTG TTT CCC ATG
 H   S   R   V   P   P   H   P   S   S   E   R   R   A   V   Y   I   G   A   L   F   P   M

AGC GGG GGC TGG CCA GGG GGC CAG GCC TGC CAG CCC GCG GTG GAG ATG GCG CTG GAG GAC GTG AAT AGC
 S   G   G   W   P   G   G   Q   A   C   Q   P   A   V   E   M   A   L   E   D   V   N   S

CGC AGG GAC ATC CTG CCG GAC TAT GAG CTC AAG CTC ATC CAC CAC GAC AGC AAG TGT GAT CCA GGC CAA
 R   R   D   I   L   P   D   Y   E   L   K   L   I   H   H   D   S   K   C   D   P   G   Q

GCC ACC AAG TAC CTA TAT GAG CTG CTC TAC AAC GAC CCT ATC AAG ATC ATC CTT ATG CCT GGC TGC AGC
 A   T   K   Y   L   Y   E   L   L   Y   N   D   P   I   K   I   I   L   M   P   G   C   S

TCT GTC TCC ACG CTG GTG GCT GAG GCT GCT AGG ATG TGG AAC CTC ATT GTG CTT TCC TAT GGC TCC AGC
 S   V   S   T   L   V   A   E   A   A   R   M   W   N   L   I   V   L   S   Y   G   S   S

TCA CCA GCC CTG TCA AAC CGG CAG CGT TTC CCC ACT TTC TTC CGA ACG CAC CCA TCA GCC ACA CTC CAC
 S   P   A   L   S   N   R   Q   R   F   P   T   F   F   R   T   H   P   S   A   T   L   H

AAC CCT ACC CGC GTG AAA CTC TTT GAA AAG TGG GGC TGG AAG AAG ATT GCT ACC ATC CAG CAG ACC ACT
 N   P   T   R   V   K   L   F   E   K   W   G   W   K   K   I   A   T   I   Q   Q   T   T

GAG GTC TTC ACT TCG ACT CTG GAC GAC CTG GAG GAA CGA GTG AAG GAG GCT GGA ATT GAG ATT ACT TTC
 E   V   F   T   S   T   L   D   D   L   E   E   R   V   K   E   A   G   I   E   I   T   F

CGC CAG AGT TTC TTC TCA GAT CCA GCT GTG CCC GTC AAA AAC CTG AAG CGC CAG GAT GCC CGA ATC ATC
 R   Q   S   F   F   S   D   P   A   V   P   V   K   N   L   K   R   Q   D   A   R   I   I

GTG GGA CTT TTC TAT GAG ACT GAA GCC CGG AAA GTT TTT TGT GAG GTG TAC AAG GAG CGT CTC TTT GGG
 V   G   L   F   Y   E   T   E   A   R   K   V   F   C   E   V   Y   K   E   R   L   F   G

AAG AAG TAC GTC TGG TTC CTC ATT GGG TGG TAT GCT GAC AAT TGG TTC AAG ATC TAC GAC CCT TCT ATC
 K   K   Y   V   W   F   L   I   G   W   Y   A   D   N   W   F   K   I   Y   D   P   S   I

AAC TGC ACA GTG GAT GAG ATG ACT GAG GCG GTG GAG GGC CAC ATC ACA ACT GAG ATT GTC ATG CTG AAT
 N   C   T   V   D   E   M   T   E   A   V   E   G   H   I   T   T   E   I   V   M   L   N

CCT GCC AAT ACC CGC AGC ATT TCC AAC ATG ACA TCC CAG GAA TTT GTG GAG AAA CTA ACC AAG CGA CTG
 P   A   N   T   R   S   I   S   N   M   T   S   Q   E   F   V   E   K   L   T   K   R   L

AAA AGA CAC CCT GAG GAG ACA GGA GGC TTC CAG GAG GCA CCG CTG GCC TAT GAT GCC ATC TGG GCC TTG
 K   R   H   P   E   E   T   G   G   F   Q   E   A   P   L   A   Y   D   A   I   W   A   L

GCA CTG GCC CTG AAC AAG ACA TCT GGA GGA GGC GGC CGT TCC GGC GTG CGC CTG GAG GAC TTC AAC TAC
 A   L   A   L   N   K   T   S   G   G   G   G   R   S   G   V   R   L   E   D   F   N   Y

AAC AAC CAG ACC ATT ACC GAC CAA ATC TAC CGG GCA ATG AAC TCC TCG TCC TTT GAG GGT GTC TCT GGC
 N   N   Q   T   I   T   D   Q   I   Y   R   A   M   N   S   S   S   F   E   G   V   S   G

CAT GTG GTG TTT GAT GCC AGC GGC TCT CGG ATG GCA TGG ACG CTT ATC GAG CAG CTT CAG GGT GGC AGC
 H   V   V   F   D   A   S   G   S   R   M   A   W   T   L   I   E   Q   L   Q   G   G   S

TAC AAG AAG ATT GGC TAC TAT GAC AGC ACC AAG GAT GAT CTT TCC TGG TCC AAA ACA GAT AAA TGG ATT
 Y   K   K   I   G   Y   Y   D   S   T   K   D   D   L   S   W   S   K   T   D   K   W   I

GGA GGG TCC CCC CCA GCT GAC CAG ACC CTG GTC ATC AAG ACA TTC CGC TTC CTG TCA CAG AAA CTC TTT
 G   G   S   P   P   A   D   Q   T   L   V   I   K   T   F   R   F   L   S   Q   K   L   F

ATC TCC GTC TCA GTT CTC TCC AGC CTG GGC ATT GTC CTA GCT GTT GTC TGT CTG TCC TTT AAC ATC TAC
 I   S   V   S   V   L   S   S   L   G   I   V   L   A   V   V   C   L   S   F   N   I   Y

AAC TCA CAT GTC CGT TAT ATC CAG AAC TCA CAG CCC AAC CTG AAC AAC CTG ACT GCT GTG GGC TGC TCA
 N   S   H   V   R   Y   I   Q   N   S   Q   P   N   L   N   N   L   T   A   V   G   C   S

CTG GCT TTA GCT GCT GTC TTC CCC CTG GGG CTC GAT GGT TAC CAC ATT GGG AGG AAC CAG TTC CCT TTC
 L   A   L   A   A   V   F   P   L   G   L   D   G   Y   H   I   G   R   N   Q   F   P   F

GTC TGC CAG GCC CGC CTC TGG CTC CTG GGC CTG GGC TTT AGT CTG GGC TAC GGT TCC ATG TTC ACC AAG
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 V   C   Q   A   R   L   W   L   L   G   L   G   F   S   L   G   Y   G   S   M   F   T   K

ATT TGG TGG GTC CAC ACG GTC TTC ACA AAG AAG GAA GAA AAG AAG GAG TGG AGG AAG ACT CTG GAA CCC
 I   W   W   V   H   T   V   F   T   K   K   E   E   K   K   E   W   R   K   T   L   E   P

TGG AAG CTG TAT GCC ACA GTG GGC CTG CTG GTG GGC ATG GAT GTC CTC ACT CTC GCC ATC TGG CAG ATC
 W   K   L   Y   A   T   V   G   L   L   V   G   M   D   V   L   T   L   A   I   W   Q   I

GTG GAC CCT CTG CAC CGG ACC ATT GAG ACA TTT GCC AAG GAG GAA CCT AAG GAA GAT ATT GAC GTC TCT
 V   D   P   L   H   R   T   I   E   T   F   A   K   E   E   P   K   E   D   I   D   V   S

ATT CTG CCC CAG CTG GAG CAT TGC AGC TCC AGG AAG ATG AAT ACA TGG CTT GGC ATT TTC TAT GGT TAC
 I   L   P   Q   L   E   H   C   S   S   R   K   M   N   T   W   L   G   I   F   Y   G   Y

AAG GGG CTG CTG CTG CTG CTG GGA ATC TTC CTT GCT TAT GAG ACC AAG AGT GTG TCC ACT GAG AAG ATC
 K   G   L   L   L   L   L   G   I   F   L   A   Y   E   T   K   S   V   S   T   E   K   I

AAT GAT CAC CGG GCT GTG GGC ATG GCT ATC TAC AAT GTG GCA GTC CTG TGC CTC ATC ACT GCT CCT GTC
 N   D   H   R   A   V   G   M   A   I   Y   N   V   A   V   L   C   L   I   T   A   P   V

ACC ATG ATT CTG TCC AGC CAG CAG GAT GCA GCC TTT GCC TTT GCC TCT CTT GCC ATA GTT TTC TCC TCC
 T   M   I   L   S   S   Q   Q   D   A   A   F   A   F   A   S   L   A   I   V   F   S   S

TAT ATC ACT CTT GTT GTG CTC TTT GTG CCC AAG ATG CGC AGG CTG ATC ACC CGA GGG GAA TGG CAG TCG
 Y   I   T   L   V   V   L   F   V   P   K   M   R   R   L   I   T   R   G   E   W   Q   S

GAG GCG CAG GAC ACC ATG AAG ACA GGG TCA TCG ACC AAC AAC AAC GAG GAG GAG AAG TCC CGG CTG TTG
 E   A   Q   D   T   M   K   T   G   S   S   T   N   N   N   E   E   E   K   S   R   L   L

GAG AAG GAG AAC CGT GAA CTG GAA AAG ATC ATT GCT GAG AAA GAG GAG CGT GTC TCT GAA CTG CGC CAT
 E   K   E   N   R   E   L   E   K   I   I   A   E   K   E   E   R   V   S   E   L   R   H

CAA CTC CAG TCT CGG CAG CAG CTC CGC TCC CGG CGC CAC CCA CCG ACA CCC CCA GAA CCC TCT GGG GGC
 Q   L   Q   S   R   Q   Q   L   R   S   R   R   H   P   P   T   P   P   E   P   S   G   G

CTG CCC AGG GGA CCC CCT GAG CCC CCC GAC CGG CTT AGC TGT GAT GGG AGT CGA GTG CAT TTG CTT TAT
 L   P   R   G   P   P   E   P   P   D   R   L   S   C   D   G   S   R   V   H   L   L   Y

AAG TGA
 K   Stp
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GABBR2

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                                                                ATG GCT TCC CCG CCT CCC GCA CTC AGC TCG CTC CCA
                                                                  M   A   S   P   P   P   A   L   S   S   L   P

CCC CTT CCC GGC GTG ATT GGT CCG TCA CGG GCG CCG CCG CCG CCA CCG CCG CCC GCG CGC CTG CTA CTG
 P   L   P   G   V   I   G   P   S   R   A   P   P   P   P   P   P   P   A   R   L   L   L

CTA CTG CTG CTG CCG CTG CTG CTG CCT CTG GCG CCC GGG GCC TGG GGC TGG GCG CGG GGC GCC CCC CGG
 L   L   L   L   P   L   L   L   P   L   A   P   G   A   W   G ▼ W   A   R   G   A   P   R
                                                                Predicted native signal sequence protease cleavage site

CCG CCG CCC AGC AGC CCG CCG CTC TCC ATC ATG GGC CTC ATG CCG CTC ACC AAG GAG GTG GCC AAG GGC
 P   P   P   S   S   P   P   L   S   I   M   G   L   M   P   L   T   K   E   V   A   K   G

AGC ATC GGG CGC GGT GTG CTC CCC GCC GTG GAA CTG GCC ATC GAG CAG ATC CGC AAC GAG TCA CTC CTG
 S   I   G   R   G   V   L   P   A   V   E   L   A   I   E   Q   I   R   N   E   S   L   L

CGC CCC TAC TTC CTC GAC CTG CGG CTC TAT GAC ACG GAG TGC GAC AAC GCA AAA GGG TTG AAA GCC TTC
 R   P   Y   F   L   D   L   R   L   Y   D   T   E   C   D   N   A   K   G   L   K   A   F

TAC GAT GCA ATA AAA TAC GGG CCG AAC CAC TTG ATG GTG TTT GGA GGC GTC TGT CCA TCC GTC ACA TCC
 Y   D   A   I   K   Y   G   P   N   H   L   M   V   F   G   G   V   C   P   S   V   T   S

ATC ATT GCA GAG TCC CTC CAA GGC TGG AAT CTG GTG CAG CTT TCT TTT GCT GCA ACC ACG CCT GTT CTA
 I   I   A   E   S   L   Q   G   W   N   L   V   Q   L   S   F   A   A   T   T   P   V   L

GCC GAT AAG AAA AAA TAC CCT TAT TTC TTT CGG ACC GTC CCA TCA GAC AAT GCG GTG AAT CCA GCC ATT
 A   D   K   K   K   Y   P   Y   F   F   R   T   V   P   S   D   N   A   V   N   P   A   I

CTG AAG TTG CTC AAG CAC TAC CAG TGG AAG CGC GTG GGC ACG CTG ACG CAA GAC GTT CAG AGG TTC TCT
 L   K   L   L   K   H   Y   Q   W   K   R   V   G   T   L   T   Q   D   V   Q   R   F   S

GAG GTG CGG AAT GAC CTG ACT GGA GTT CTG TAT GGC GAG GAC ATT GAG ATT TCA GAC ACC GAG AGC TTC
 E   V   R   N   D   L   T   G   V   L   Y   G   E   D   I   E   I   S   D   T   E   S   F

TCC AAC GAT CCC TGT ACC AGT GTC AAA AAG CTG AAG GGG AAT GAT GTG CGG ATC ATC CTT GGC CAG TTT
 S   N   D   P   C   T   S   V   K   K   L   K   G   N   D   V   R   I   I   L   G   Q   F
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GAC CAG AAT ATG GCA GCA AAA GTG TTC TGT TGT GCA TAC GAG GAG AAC ATG TAT GGT AGT AAA TAT CAG
 D   Q   N   M   A   A   K   V   F   C   C   A   Y   E   E   N   M   Y   G   S   K   Y   Q

TGG ATC ATT CCG GGC TGG TAC GAG CCT TCT TGG TGG GAG CAG GTG CAC ACG GAA GCC GAC TCA TCC CGC
 W   I   I   P   G   W   Y   E   P   S   W   W   E   Q   V   H   T   E   A   D   S   S   R

TGC CTC CGG AAG AAT CTG CTT GCT GCC ATG GAG GGC TAC ATT GGC GTG GAT TTC GAG CCC CTG AGC TCC
 C   L   R   K   N   L   L   A   A   M   E   G   Y   I   G   V   D   F   E   P   L   S   S

AAG CAG ATC AAG ACC ATC TCA GGA AAG ACT CCA CAG CAG TAT GAG AGA GAG TAC AAC AAC AAG CGG TCA
 K   Q   I   K   T   I   S   G   K   T   P   Q   Q   Y   E   R   E   Y   N   N   K   R   S

GGC GTG GGG CCC AGC AAG TTC CAC GGG TAC GCC TAC GAT GGC ATC TGG GTC ATC GCC AAG ACA CTG CAG
 G   V   G   P   S   K   F   H   G   Y   A   Y   D   G   I   W   V   I   A   K   T   L   Q

AGG GCC ATG GAG ACA CTG CAT GCC AGC AGC CGG CAC CAG CGG ATC CAG GAC TTC AAC TAC ACG GAC CAC
 R   A   M   E   T   L   H   A   S   S   R   H   Q   R   I   Q   D   F   N   Y   T   D   H

ACG CTG GGC AGG ATC ATC CTC AAT GCC ATG AAC GAG ACC AAC TTC TTC GGG GTC ACG GGT CAA GTT GTA
 T   L   G   R   I   I   L   N   A   M   N   E   T   N   F   F   G   V   T   G   Q   V   V

TTC CGG AAT GGG GAG AGA ATG GGG ACC ATT AAA TTT ACT CAA TTT CAA GAC AGC AGG GAG GTG AAG GTG
 F   R   N   G   E   R   M   G   T   I   K   F   T   Q   F   Q   D   S   R   E   V   K   V

GGA GAG TAC AAC GCT GTG GCC GAC ACA CTG GAG ATC ATC AAT GAC ACC ATC AGG TTC CAA GGA TCC GAA
 G   E   Y   N   A   V   A   D   T   L   E   I   I   N   D   T   I   R   F   Q   G   S   E

CCA CCA AAA GAC AAG ACC ATC ATC CTG GAG CAG CTG CGG AAG ATC TCC CTA CCT CTC TAC AGC ATC CTC
 P   P   K   D   K   T   I   I   L   E   Q   L   R   K   I   S   L   P   L   Y   S   I   L

TCT GCC CTC ACC ATC CTC GGG ATG ATC ATG GCC AGT GCT TTT CTC TTC TTC AAC ATC AAG AAC CGG AAT
 S   A   L   T   I   L   G   M   I   M   A   S   A   F   L   F   F   N   I   K   N   R   N

CAG AAG CTC ATA AAG ATG TCG AGT CCA TAC ATG AAC AAC CTT ATC ATC CTT GGA GGG ATG CTC TCC TAT
 Q   K   L   I   K   M   S   S   P   Y   M   N   N   L   I   I   L   G   G   M   L   S   Y

GCT TCC ATA TTT CTC TTT GGC CTT GAT GGA TCC TTT GTC TCT GAA AAG ACC TTT GAA ACA CTT TGC ACC
 A   S   I   F   L   F   G   L   D   G   S   F   V   S   E   K   T   F   E   T   L   C   T

GTC AGG ACC TGG ATT CTC ACC GTG GGC TAC ACG ACC GCT TTT GGG GCC ATG TTT GCA AAG ACC TGG AGA
 V   R   T   W   I   L   T   V   G   Y   T   T   A   F   G   A   M   F   A   K   T   W   R

GTC CAC GCC ATC TTC AAA AAT GTG AAA ATG AAG AAG AAG ATC ATC AAG GAC CAG AAA CTG CTT GTG ATC
 V   H   A   I   F   K   N   V   K   M   K   K   K   I   I   K   D   Q   K   L   L   V   I

GTG GGG GGC ATG CTG CTG ATC GAC CTG TGT ATC CTG ATC TGC TGG CAG GCT GTG GAC CCC CTG CGA AGG
 V   G   G   M   L   L   I   D   L   C   I   L   I   C   W   Q   A   V   D   P   L   R   R

ACA GTG GAG AAG TAC AGC ATG GAG CCG GAC CCA GCA GGA CGG GAT ATC TCC ATC CGC CCT CTC CTG GAG
 T   V   E   K   Y   S   M   E   P   D   P   A   G   R   D   I   S   I   R   P   L   L   E

CAC TGT GAG AAC ACC CAT ATG ACC ATC TGG CTT GGC ATC GTC TAT GCC TAC AAG GGA CTT CTC ATG TTG
 H   C   E   N   T   H   M   T   I   W   L   G   I   V   Y   A   Y   K   G   L   L   M   L

TTC GGT TGT TTC TTA GCT TGG GAG ACC CGC AAC GTC AGC ATC CCC GCA CTC AAC GAC AGC AAG TAC ATC
 F   G   C   F   L   A   W   E   T   R   N   V   S   I   P   A   L   N   D   S   K   Y   I

GGG ATG AGT GTC TAC AAC GTG GGG ATC ATG TGC ATC ATC GGG GCC GCT GTC TCC TTC CTG ACC CGG GAC
 G   M   S   V   Y   N   V   G   I   M   C   I   I   G   A   A   V   S   F   L   T   R   D

CAG CCC AAT GTG CAG TTC TGC ATC GTG GCT CTG GTC ATC ATC TTC TGC AGC ACC ATC ACC CTC TGC CTG
 Q   P   N   V   Q   F   C   I   V   A   L   V   I   I   F   C   S   T   I   T   L   C   L

GTA TTC GTG CCG AAG CTC ATC ACC CTG AGA ACA AAC CCA GAT GCA GCA ACG CAG AAC AGG CGA TTC CAG
 V   F   V   P   K   L   I   T   L   R   T   N   P   D   A   A   T   Q   N   R   R   F   Q

TTC ACT CAG AAT CAG AAG AAA GAA GAT TCT AAA ACG TCC ACC TCG GTC ACC AGT GTG AAC CAA GCC AGC
 F   T   Q   N   Q   K   K   E   D   S   K   T   S   T   S   V   T   S   V   N   Q   A   S

ACA TCC CGC CTG GAG GGC CTA CAG TCA GAA AAC CAT CAC CTG CGA ATG AAG ATC ACA GAG CTG GAT AAA
 T   S   R   L   E   G   L   Q   S   E   N   H   H   L   R   M   K   I   T   E   L   D   K

GAC TTG GAA GAG GTC ACC ATG CAG CTG CAG GAC ACA CCA GAA AAG ACC ACC TAC ATT AAA CAG AAC CAC
 D   L   E   E   V   T   M   Q   L   Q   D   T   P   E   K   T   T   Y   I   K   Q   N   H

TAC CAA GAG CTC AAT GAC ATC CTC AAC CTG GGA AAC TTC ACT GAG AGC ACA GAT GGA GGA AAG GCC ATT
 Y   Q   E   L   N   D   I   L   N   L   G   N   F   T   E   S   T   D   G   G   K   A   I

TTA AAA AAT CAC CTC GAT CAA AAT CCC CAG CTA CAG TGG AAC ACA ACA GAG CCC TCT CGA ACA TGC AAA
 L   K   N   H   L   D   Q   N   P   Q   L   Q   W   N   T   T   E   P   S   R   T   C   K

GAT CCT ATA GAA GAT ATA AAC TCT CCA GAA CAC ATC CAG CGT CGG CTG TCC CTC CAG CTC CCC ATC CTC
 D   P   I   E   D   I   N   S   P   E   H   I   Q   R   R   L   S   L   Q   L   P   I   L

CAC CAC GCC TAC CTC CCA TCC ATC GGA GGC GTG GAC GCC AGC TGT GTC AGC CCC TGC GTC AGC CCC ACC
 H   H   A   Y   L   P   S   I   G   G   V   D   A   S   C   V   S   P   C   V   S   P   T

GCC AGC CCC CGC CAC AGA CAT GTG CCA CCC TCC TTC CGA GTC ATG GTC TCG GGC CTG TGA
 A   S   P   R   H   R   H   V   P   P   S   F   R   V   M   V   S   G   L  Stp
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PRODUCT NUMBER	DESCRIPTION
HTSCHEM-1RTA	Ready-to-Assay™ Chem-1 host frozen cells (control cells)
HTS119M	ChemiScreen™ $GABA_B$ GABA Family Receptor membrane prep

REFERENCES

1. Bowery NG *et al.* (2002) International Union of Pharmacology. XXXIII. Mammalian y-aminobutyric $acid_B$ receptors: Structure and function. *Pharmacol. Rev.* 54: 247-264.

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