PRODUCT DATASHEET

ChemiScreen™ GABA$_B$ GABA Family Receptor Stable Cell Line

CATALOG NUMBER: HTS119C

CONTENTS: 2 vials of mycoplasma-free cells, 1 ml per vial.

STORAGE: Vials are to be stored in liquid N$_2$.

BACKGROUND

ChemiScreen cell lines are constructed in the Chem-1 host, which supports high levels of functional receptor expression on the cell surface. Chem-1 cells contain high endogenous levels of Gα15, a promiscuous G protein, allowing most receptors to couple to the calcium signaling pathway.

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$_{B1b}$/GABA$_{B2}$ receptor-expressing ChemiScreen cells were constructed by stable transfection of Chem-1 cells with GABA$_{B1b}$/GABA$_{B2}$ which supports high levels of recombinant GABA$_{B1b}$/GABA$_{B2}$ expression on the cell surface and contains high levels of the promiscuous G protein Gα15 to couple the receptor to the calcium signaling pathway. These stability-tested cells are ready for fluorescence-based assays for agonists, antagonists and modulators at the GABA$_B$ receptor.

USE RESTRICTIONS

Please see **Limited Use Label License Agreement** (Label License Agreement) for further details.

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.

APPLICATIONS

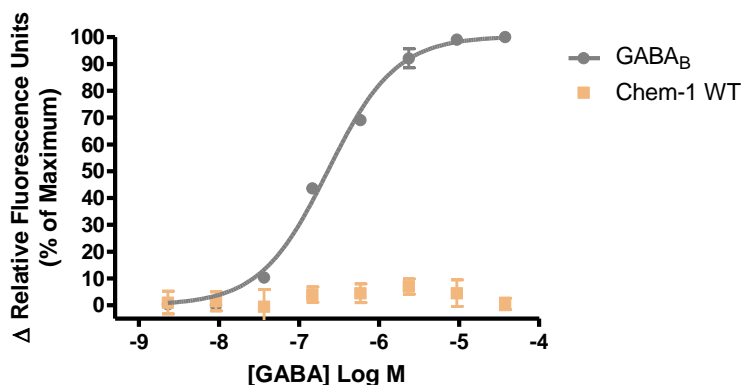Calcium Flux Fluorescence Assay

APPLICATION DATA

Figure 1. Representative data for activation of $GABA_{B1b}$/$GABA_{B2}$ receptor stably expressed in Chem-1 cells induced by GABA using a fluorescent calcium flux assay. $GABA_{B1b}$/$GABA_{B2}$–expressing Chem-1 cells were seeded at 50,000 cells per well into a 96-well plate, and the following day the cells were loaded with a fluorescent calcium indicator. Calcium flux in response to the indicated ligand with a final concentration of 0.5% DMSO was determined on a Molecular Devices FLIPR^{TETRA}® with ICCD camera. Maximal fluorescence signal obtained in this experiment was 5,000 RLU. Similarly parental cells (catalog #: HTSCHEM-1) were tested to determine the specificity of the resulting signal.

Table 1. EC_{50} values of $GABA_{B1b}$/$GABA_{B2}$-expressing Chem-1 cells.

LIGAND	ASSAY	POTENCY EC_{50} (nM)	REFERENCE
GABA	Calcium Flux - Fluorescence	230	Eurofins Internal Data

* The cell line was tested and found to have equivalent EC_{50} and signal at 1, 3 and 6 weeks of continuous culture by calcium flux fluorescence. The Z' value, as defined with response to GABA, was 0.7.

CELL CULTURE

Table 2. Recommended Cell Culture Reagents (not provided)

Description	Component	Concentration	Supplier and Product Number
Basal Medium	DMEM high glucose Medium (4.5g/L)	-	Hyclone: SH30022
	Fetal Bovine Serum (FBS)	10%	Hyclone: SH3007003
	Non-Essential Amino Acids (NEAA)	1X	Hyclone: SH3023801
	HEPES	1X	EMD Millipore:TMS-003-C
Selection Medium	Basal Medium (see above)	-	
	Geneticin (G418)	250 µg/ml	Invivogen: ant-gn-5
	Hygromycin	500 µg/ml	Invivogen: ant-hm-5
Dissociation	Sterile PBS	-	Hyclone: SH3025601
	0.25% Trypsin-EDTA	-	Hyclone: SH3004201
CryoMedium	Basal Medium (see above)	40%	-
	Fetal Bovine Serum (FBS)	50%	Hyclone: SH3007003
	Dimethyl Sulfoxide (DMSO)	10%	Sigma: D2650

Cell handling

1. Upon receipt, directly place cells in liquid nitrogen storage. Consistent cryopreservation is essential for culture integrity.
2. Prepare Basal Medium. Prepare 37ºC Water Bath. Thaw cells rapidly by removing from liquid nitrogen, and immediately immersing in a 37ºC water bath, until 90% thawed. Immediately sterilize the exterior of the vial with 70% ethanol.
3. Add vial contents to 15 mL Basal Medium in T75 Tissue Culture Treated Flask. Gently swirl flask and place in a humidified, tissue culture incubator, 37ºC, 5% CO_2.
4. 18-24 Hours Post–Thaw, all live cells should be attached. Viability of the cells is expected to be 60-90%, at this time, exchange Basal Medium with Selection Medium.
5. When cells are approximately 80% confluent, passage the cells. It is suggested that user expand culture to create >20 vial Master Cell Bank at low passage number. *Cells should be maintained at less than 80% confluency for optimal assay results.*
6. Cell Dissociation: Aspirate Culture Medium. Gently wash with 1x Volume PBS. Add 0.1x Volume Warm Trypsin-EDTA. Incubate 4 min, 37°C, until cells dislodge. *If cells do not round up, place in 37º C incubator for additional 2 min.* Neutralize Trypsin and collect cells in 1x Volume Basal Medium.
7. Seed Cells for expansion of culture. It is recommended that cell lines are passaged at least once before use in assays.

Table 3. Cell Culture Seeding Suggestions: *User should define based on research needs.*

Flask Size (cm^2)	Volume (mL)	Total Cell Number ($x10^6$)	Growth Period (hrs)
T75	15	5.0	24
T75	15	2.0	48
T75	15	0.45	72

ASSAY SETUP
Fluorescence

Table 4. 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	95 µl (50 µl for 384-well)
Dispense Speed	50 µl L/sec
Expel Volume	0 µl
Analysis	Subtract Bias Sample 1

Table 5. Assay Materials (Not provided)

Description	Supplier and Product Number
HBSS	Invitrogen: 14025
HEPES 1M Stock	EMD Millipore: TMS-003-C
Probenicid	Sigma:P8761
Quest Fluo-8™, AM	AAT Bioquest: 21080
GABA ligand	Tocris: 0344
Non-Binding 96/384 well Plates (for ligand prep)	Corning: 3605/ 3574
Black (clear Bottom) cell assay plates	Corning: 3904/ 3712

Assay Protocol - Fluorescence

1.   Dissociate Culture as Recommended. Collect in Basal Medium. Document Cell Count and Viability

2.   Centrifuge the cell suspension at 190 x g for six min

3.   Remove supernatant. Gently resuspend the cell pellet in Basal Medium. *It is suggested that end user optimize cell plating based on individual formats.* (Default: Resuspend in volume to achieve 5×10^5 cells/ml *(i.e, if collected 5e6 TC, $^{5e6/}_{5e5/ml}$ =10 mL volume)*

4.   Seed cell suspension into black, clear bottom plate (100 µL/well for 96-well plate). *When seeding is complete, place the assay plate at room temperature for 30 min.*

5.   Move assay plate to a humidified 37ºC 5% CO_2 incubator for 18-24 h.

6.   Next day, prepare Assay buffer (HBSS, 20mM HEPES, 2.5 mM Probenicid, pH 7.4) and Loading buffer (Assay buffer with 5 mM Fluo8 Dye). *Note: Please prepare Fluo8 stock according to Manufacturer's Recommendations*

7.   Remove medium from assay plate and wash 1X with Assay Buffer.

8.   Add Loading buffer to assay plate (100 µL/well for 96-well plate). Incubate plate for 1.5 h at room temperature, protected from light.

9.   Prepare ligands in assay buffer at 3x final concentration in non-binding plates. Use Buffer Only Control Wells for Background Subtraction.

10.   Create protocol for ligand addition. Please refer to FLIPR^TETRA® settings provided in Table 2. Set time course for 180 s, with ligand addition at 10 s.

11.   After the run is complete, apply subtract bias on sample 1. We recommend using Negative Control Correction with Buffer Only Wells. Export data to according to research needs. For most Calcium Flux analysis using Export of Max Signal to end of run is sufficient.

HOST CELL

Chem-1, an adherent cell line expressing the promiscuous G-protein, Gα15.

EXOGENOUS 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
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                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
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  AGG  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  GGG  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
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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

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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