

Commonly Used Reagents

Revised on June 14, 2016

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Solubility advice:

Some solutions can be difficult to obtain and can be encouraged by rapid stirring, sonication or gentle warming (in a 45-60°C water bath).

- 100 mM acetylcholine chloride (SIGMA, A2661-25G)

Dissolve 18.2 mg of acetylcholine chloride in 1.0 ml of appropriate buffer (BRET buffer). Store at -20°C.

- 100 mM adenylyl-imidodiphosphate, tetralithium salt (AppNHp) (Calbiochem, 120002)

Dissolve 25 mg of AppNHp in 472 µl of H₂O. Store at -20°C.

Note:

1. A non-hydrolyzable ATP analog that acts as a competitive inhibitor of ATP-dependent enzyme systems. Known to block ATP-sensitive Ca²⁺-activated K⁺ channels.
2. Molecular Weight: 529.9
3. CAS Number: 72957-42-7

- 100 mM (-)-adrenaline ((-)-epinephrine) (Sigma, E4250-1G)

Dissolve 18.32 mg of (-)-adrenaline (100 mM) in 1 ml of H₂O or appropriate buffer and add HCl to a final concentration of 0.1 N HCl. Store at -20°C.

Note: Wherever possible, you should prepare and use solutions on the same day.

1. Molecular Weight: 183.2
2. CAS Number: 51-43-4

- 0.2 mM angiotensin II (Tocris, 1158)

Dissolve 5 mg of angiotensin II in 23.896 ml of H₂O or appropriate buffer. Store at -20°C.

- Antibiotic Solution

	Stock Solution ^a		Working Concentration	
	Concentration	Storage	Stringent Plasmids	Relaxed Plasmids
Ampicillin ^c	50 mg/ml in H ₂ O	-20°C	20 µg/ml	50 µg/ml
Carbenicillin (disodium salt)	50 mg/ml in H ₂ O	-20°C for up to four months	20 µg/ml	60 µg/ml
Chloramphenicol	34 mg/ml in ethanol	-20°C	25 µg/ml	170 µg/ml
Kanamycin	50 mg/ml in H ₂ O	-20°C	10 µg/ml	50 µg/ml
Streptomycin	10 mg/ml in H ₂ O	-20°C	10 µg/ml	50 µg/ml
Tetracycline ^b	5 mg/ml in ethanol	-20°C	10 µg/ml	50 µg/ml

Magnesium ions are antagonists of tetracycline. Use media without magnesium salts (e.g. LB medium) for selection of bacteria to tetracycline.

^a Sterilize stock of antibiotics dissolved in H₂O by filtration through a 0.22- μ m filter.

^b Antibiotics dissolved in ethanol need not be sterilized. Store solutions in light-tight containers.

^c Ampicillin sodium salt (CAS 69-52-3) is soluble in H₂O.

· 10% ammonium persulfate (Fisher Scientific, BP179-100)

Dissolve 2 g of ammonium persulfate (APS) in 15 ml of H₂O. Adjust the volume of the solution to 20 ml with H₂O. Make 1 ml aliquots and store at -20°C.

Note: CAS Number: 7727-54-0

· 100 mM atropine (SIGMA, A0257)

Dissolve 695 mg of atropine sulfate salt monohydrate (100 mM) in 10 ml of H₂O or appropriate buffer. Store at -20°C.

Note:

1. Competitive nonselective antagonist at central and peripheral muscarinic acetylcholine receptors.
2. Molecular Weight: 694.83
3. CAS Number: 5908-99-6
4. Stable for several days at 4 °C.

· 100 mM R-(+)-baclofen hydrochloride (Enzo Life Sciences, ALX-550-074)

Dissolve 25.02 mg of R-(+)-baclofen hydrochloride in 1.0 ml of H₂O. Store at -20°C.

· 0.3 mM (D-Arg⁰,Hyp³,Igl⁵,D-Igl⁷,Oic⁸)-Bradykinin trifluoroacetate salt (B-9430) (BACHEM, H-7556)

Dissolve 1 mg of B-9430 in 2.4903 ml of BRET buffer. Store at -20°C.

Note:

1. Bradykinin antagonist B-9430 shows high affinity for both bradykinin receptors.
2. Molecular Weight: 1338.58

· 0.2 mM [Des-Arg⁹]-Bradykinin (Tocris, 3005)

Dissolve 5 mg of [Des-Arg⁹]-Bradykinin in 27.6539 ml of BRET buffer. Store at -20°C.

Note:

1. Bradykinin receptor agonist that displays selectivity for B1 over B2 receptors.

· 30 mM BAPTA-AM (Tocris, 2787)

Dissolve 25 mg of BAPTA-AM in 1.09 ml of DMSO. Vortex is required to dissolve the powder. Store at -20°C.

· 100 mM β -estradiol (Tocris, 2824)

Dissolve 100 mg of β -estradiol in 3.5 ml of DMSO. Store stock solutions in light-tight containers at -20°C .

· 30 mM BQCA (Sigma, SML0497-10MG)

Dissolve 10 mg of BQCA in 1.0776 ml of DMSO. Store at -20°C .

· 0.2 mM bradykinin (Tocris, 3004) or (Calbiochem, 05-23-0500-5MG)

Dissolve 5 mg of bradykinin in 23.58 ml of appropriate buffer. Store at -20°C .

Note: Endogenous bradykinin receptor agonist that displays selectivity for B_2 over B_1 receptors. Proinflammatory peptide.

· 100 mM 8-Bromo-cAMP (Tocris, 1140)

Dissolve 10 mg of 8-Bromo-cAMP, sodium salt in 233 μl of H_2O or BRET buffer. Store at -20°C .

Note:

1. Cell-permeable cAMP analog; activator of protein kinase A.

· 12.5 mM buprenorphine hydrochloride (SIGMA, B9275-50MG)

Add 2 ml of ethanol and 2 ml of BRET buffer to dissolve 25.21 mg of buprenorphine hydrochloride. Store at -20°C .

Note:

1. ORL_1 receptor agonist that also displays mixed antagonist/partial agonist activity at κ , δ and μ -opioid receptors.
2. Molecular Weight: 504.1
3. CAS Number: 53152-21-9
4. Solubility: 25 mg/ml, EtOH

· 1 M CaCl_2

Dissolve 5.5495 g of CaCl_2 in 30 ml of H_2O and add distilled H_2O to 50 ml. Store at room temperature.

· 0.2 mM calcitonin (Abcam, ab142264)

Dissolve 1 mg of calcitonin in 1.4629 ml of appropriate buffer. Store at -20°C .

· 0.2 mM CCK octapeptide sulfated (CCK-8) (Abcam, ab120209)

Dissolve 1 mg of CCK octapeptide in 4.3733 ml of appropriate buffer. Sonicate briefly to dissolve, and avoid heating. Store at -20°C .

· 10% CHAPS (SIGMA, C3023)

Take 5 g of CHAPS into a 50-ml tube, add H_2O to 50 ml, and shake at room temperature until dissolve. Store at 4°C . Stock solutions are stable for up to 6 months at 4°C .

· 1,000 x carbenicillin

Dissolve 3 g of carbenicillin disodium salt (60 mg/ml) (Fisher, BP2648-5) in 30 ml of H₂O and add distilled H₂O to 50 ml. Sterilize stock of antibiotics dissolved in H₂O by filtration through a 0.22- μ m filter. Make 1 ml aliquots and store solutions in light-tight containers at -20°C.

· 100 mM carbachol

Dissolve 18.3 mg carbamoylcholine chloride (100 mM) in 1 ml of H₂O or appropriate buffer.

Note:

1. Molecular Weight: 182.65
2. CAS Number: 51-83-2

· 100 mM carvedilol (Tocris, 2685)

Dissolve 50 mg of carvedilol (100 mM) in 1.23 ml of ethanol at 60°C. Store at 4°C.

Note: Potent β -adrenoceptor and α_1 -adrenoceptor antagonist (K_i values are 0.81, 0.96 and 2.2 nM for β_1 -, β_2 - and α_1 -adrenoceptors respectively) that displays antihypertensive and peripheral vasodilatory activity. Blocks cardiac inward-rectifier K⁺ (K_{IR}) channels, voltage-dependent Ca²⁺ channels and exhibits antioxidant properties at higher concentrations. **Could not prepare 30 μ M carvedilol in BRET buffer.**

· 100 mM clenbuterol hydrochloride (SIGMA, C5423-50MG)

Dissolve 50 mg of acetylcholine chloride in 1.5941 ml of appropriate buffer (BRET buffer). Store at -20°C.

· 100 mg/ml chloramphenicol in ethanol: isopropanol (95:5) (Sigma, R4408)

Used as a selection agent for transformed cells containing chloramphenicol resistance genes.

· 100 mM clocinnamox mesylate (Tocris, 0898)

Dissolve 2 mg clocinnamox mesylate (100 mM) in 33.3 μ l of DMSO. Store at -20°C.

· Complete, EDTA-free (Roche, 11 873 580 001)

One tablet Complete is sufficient for the inhibition of the proteolytic activity in 50 ml extraction solution. Prepare 100x stock solutions in 0.5 ml water. Stock solution is stable for 1-2 weeks at 2-8°C or at least 12 weeks at -15 to -25°C.

· Coomassie G-250 (Sigma, B0770-25G)

<u>Substances</u>	<u>Final conc.</u>	<u>(/1 L)</u>
Coomassie Brilliant Blue G-250	500 mg/L	500 mg
Ethanol	5.0%	50 ml
60% HClO ₄	2.0%	100 ml
H ₂ O	N/A	up to 1 L

Procedure

1. Add 25 ml of ethanol to a 100-ml beaker.
2. Add 500 mg of Coomassie Brilliant Blue G-250 into the beaker and mix for 5 min.
3. Transfer the solution into a 1-L bottle.
4. Wash the beaker with 25 ml of ethanol and transfer to the bottle.
5. Wash the beaker with H₂O and transfer to the bottle.
6. Bring volume to ~800 ml with H₂O.
7. Add 100 ml of 60% HClO₄, then titrate until the color of solution turns to reddish brown.
8. Bring volume to 1 L with H₂O.

· 50 mM CGP 54626 hydrochloride (Toctis, 1088)

Dissolve 10 mg of CGP 54626 hydrochloride in 449.6 µl of DMSO. Store at -20°C.

Note: A potent, selective GABA_B receptor antagonist (IC₅₀ = 4 nM).

· 0.1 M CPPG (TOCRIS bioscience, 0972)

Dissolve 20.0 mg of CPPG in 73.7 µl of 1 N NaOH and add appropriate buffer (BRET buffer) to 500 µl. Adjust pH to around 7.0 with 10N NaOH: monitor the pH using pH strip papers. Add buffer to 737 µl. Store at -20°C.

· 10% C₁₂E₁₀ (decaethylene glycol monododecyl ether) (SIGMA, P9769-500G)

Take 5 g of C₁₂E₁₀ into a 50-ml tube, add H₂O to 50 ml, and shake at room temperature until dissolve. Store at 4°C.

Note: Molecular weight is 626.86. CMC is 92 mM (5.767%)

· 100 mM cyprodime (Tocris, 2601)

Dissolve 10 mg of cyprodime hydrochloride in 0.255 ml of DMSO. Store at -20°C.

Note:

1. Selective µ-opioid receptor antagonist (K_i values are 5.4, 244.6 and 2187 nM for µ-, δ- and κ-opioid receptors respectively). Reduces levodopa-induced dyskinesia in the MPTP-lesioned primate model of Parkinson's disease.

· 0.2 mM [D-Ala²]-deltorphan II (Tocris, 1180)

Dissolve 1 mg of [D-Ala²]-deltorphan II in 6.387 ml of H₂O or appropriate buffer. Sonication might be necessary. Store at -20°C.

· 5 mM DAMGO ([D-Ala², N-Me-Phe⁴, Gly⁵-ol]-Enkephalin acetate salt) (SIGMA, E7384-10MG)

Dissolve 10.0 mg of DAMGO (5 mM) in 3.894 ml of H₂O or appropriate buffer. Store at -20°C.

Note: It might be possible to make 10 mM stock solution.

· 0.3 mM (D-Arg⁰,Hyp³,D-Phe⁷)-Bradykinin trifluoroacetate (BACHEM, H-6385)

Dissolve 5.0 mg of (D-Arg⁰,Hyp³,D-Phe⁷)-Bradykinin trifluoroacetate (0.3 mM) in 12.9965 ml of appropriate buffer. Store at -20°C.

Note:

1. Molecular Weight: 1282.47
2. Potent bradykinin antagonist.

· 100 mM devazepide (Tocris, 2304)

Dissolve 10.0 mg of devazepide in 0.245 ml of DMSO. Store at -20°C.

Note:

1. Potent, orally active CCK₁ (CCK-A) receptor antagonist that displays appetite-stimulant effects. Blocks the anorectic response to CCK-8 and increases food intake in rats following systemic and i.c.v administration.
2. **Cannot prepare 90 µM devazepide in BRET buffer. Difficult to dissolve.**

· 20 mM digitonin (Acros Organics, 407565000)

Dissolve 12.29 mg of digitonin in 500 µl of H₂O. Store at 4°C.

Note:

1. Molecular Weight: 1229.33
2. CAS Number: 11024-24-1
3. Digitonin is a steroid glycoside from *Digitalis purpurea*. It may permeabilize plasma membranes and may form complexes with cholesterol. It belongs to the class of non-ionic detergents and is frequently used to dissolve membrane-bound proteins. **At concentrations between 10 - 100 µg/ml, cholesterol-rich plasma membranes are permeabilized only, but not those of organelles.** Digitonin is suitable to e.g. isolate the opioid receptors from membranes of rat brains, maintaining the capability to bind different agonisten/antagonists. Digitonin is hardly soluble in either water, chloroform or ether. You may dissolve 1 g/57 ml in absolute ethanol and 1 g/220 ml in 95% ethanol, respectively. Stock solutions of digitonin (e.g. 10 % w/v or 1 mg/ml) in water or buffer (e.g. pH 7.2 - 7.5) may be prepared by heating (95 - 100°C) or vortexing, until a clear solution is obtained. Digitonin that may precipitate after cooling (+4°C) is removed by filtration. DMSO may be used as well (stock solution 20 mg/ml). Stability of stock solutions: **solutions of digitonin may be stored up to one week at +4°C.** The capability of digitonin to permeabilize membranes may differ from batch to batch from all suppliers.

· 25 mM diprenorphine (Tocris, 2965)

Dissolve 10.639 mg of diprenorphine in 1 ml of ethanol. Store at -20°C.

Reference: Molecular Pharmacology 1994, 45, 2330-334.

· 50 mM domperidone (Tocris, 2536)

Dissolve 50 mg of domperidone in 2.3479 ml of DMSO. Solutions must be protected from light. Store at -20°C.

- 50 mM dopamine hydrochloride (SIGMA, H8502)

Dissolve 94.8 mg of dopamine hydrochloride (50 mM) and 95.1 mg of sodium metabisulfite (50 mM) (SIGMA, 255556) in 10 ml of H₂O or appropriate buffer. Sodium metabisulfite was added to prevent oxidation. Solutions must be protected from light. This stock solution of dopamine should be prepared on each day of use, and then diluted in the appropriate solution immediately before use.

- 1 M DTT (SIGMA, D0632)

Weight out 1.54 g of DTT (DL-dithiothreitol) and bring to 10 ml with H₂O. Make 1 ml aliquots. Freeze and store at -20°C.

Note: CAS 3483-12-3; 0.1 M DTT, 280 nm < 0.08, 260 nm < 0.40

Some proteins get on running SDS-PAGE analysis an extra band with twice mol. wt , even when using 2-mercaptoethanol reduction. Switch to DTT reduction, or even better to DTT will achieve more complete reduction. A standard loading buffer contains 1% SDS, 10% glycerol, 10 mM Tris-Cl, pH 6.8, 1 mM EDTA, bromophenol blue tracking dye ~0.05 mg/ml and 10mM dithiothreitol (DTT) as reducing agent.

- 0.2 mM dynorphin A (abcam, ab120412)

Dissolve 1 mg of dynorphin A in 2.097 ml of appropriate buffer. Store at -20°C.

- 0.2 mM endomorphin-1 (Tocris, 1055)

Dissolve 5 mg of endomorphin-1 (0.2 mM) in 40.939 ml of appropriate buffer. Store at -20°C.

Note: Endogenous peptide with an exceptionally high affinity ($K_i = 360$ pM) and selectivity for μ opioid receptors (4000- and 15000-fold preference over δ and κ respectively).

- 1 mM [Lue⁵]-enkephalin (Tocris, 1889)

Dissolve 25 mg of [Lue⁵]-enkephalin (1 mM) in 44.994 ml of appropriate buffer. Store at -20°C.

Note:

1. It is possible to prepare higher concentration of stock solution.

- 100 mM Forskolin (Tocris, 1099)

Dissolve 10 mg of forskolin in 244 μ l of DMSO. Store at -20°C.

Note:

1. Cell-permeable activator of adenylyl cyclase

- 100 mM GABA (Toctis, 0344)

Dissolve 103.12 mg of GABA in 10 ml of H₂O or appropriate buffer. Store at -20°C.

Note: Endogenous inhibitory neurotransmitter.

- 1 M Glucose

Weight out 9.01 g of sodium bicarbonate in 30 ml of water. Adjust the volume to 50 ml with H₂O. Store at room temperature.

Note:

1. Molecular Weight: 180.16
2. CAS Number: 50-99-7

· 50 mM GDP (SIGMA, G7127)

Take 25 mg of GDP into a 2-ml tube and add 0.75 ml of H₂O. Adjust pH to around 7.0 with 10N NaOH: monitor the pH using pH strip papers. You may need ~4 µl of 10N NaOH to adjust pH. Check OD₂₅₂ of a 1:1,000 dilution and determine a concentration of GDP solution.

Note: Do not use disposable cuvette. If you use the disposable cuvette, OD₂₅₂ will be unstable.

$(OD_{252} \times \text{dilution factor}) / \text{extinction coefficient} = \text{concentration}$

extinction coefficient $\epsilon_{252} = 13.700 \text{ M}^{-1}\text{cm}^{-1}$

for example: OD₂₅₂ = 0.845, then $(0.845 \times 1,000) / 13.7 = 61.7 \text{ mM}$

Dilute the solution with H₂O into a final concentration of 50 mM and store at -20°C.

· 100 mM 6'-GNTI dihydrochloride (Tocris, 2962)

Dissolve 10 mg of 6'-GNTI dihydrochloride in 183.7 µl of appropriate buffer (BRET buffer). Store at -20°C.

· 0.2 mM GRP (Tocris, 1789)

Dissolve 1 mg of gastrin-releasing peptide (GRP) in 1.7486 ml of appropriate buffer. Store at -20°C.

· 50 mM GTP (SIGMA, G8877)

Take 30 mg of GTP into a 2-ml tube and add 0.75 ml of H₂O. Adjust pH to around 7.0 with 10N NaOH: monitor the pH using pH strip papers. You may need ~4 µl of 10N NaOH to adjust pH. Check OD₂₅₂ of a 1:1,000 dilution and determine a concentration of GTP solution.

Note: Do not use disposable cuvette. If you use the disposable cuvette, OD₂₅₂ will be unstable.

$(OD_{252} \times \text{dilution factor}) / \text{extinction coefficient} = \text{concentration}$

extinction coefficient $\epsilon_{252} = 13.700 \text{ M}^{-1}\text{cm}^{-1}$

for example: OD₂₅₂ = 0.845, then $(0.845 \times 1,000) / 13.7 = 61.7 \text{ mM}$

Dilute the solution with H₂O into a final concentration of 50 mM and store at -20°C.

· 10 mM GTP γ S (SIGMA, L8895)

· 50 mM H2L5186303 (Tocris, 4878)

Dissolve 10 mg of H2L5186303 in 409.5 µl of DMSO. Stored at -20°C.

Note:

1. Potent and selective lysophosphatidic acid 2 (LPA₂) receptor antagonist (IC₅₀ values are 8.9, 1230 and 27354 nM for LPA₂, LPA₃ and LPA₁ receptors respectively, in a LPA-elicited calcium mobilization

- assay).
2. Molecular Weight: 488.45
 3. CAS Number: 139262-76-3
- 50 mM haloperidol (SIGMA, H1512-5G)

Dissolve 18.8 mg of haloperidol in 1 ml of DMSO. Stored at -20°C.

Biological activity from Tocris: Dopamine antagonist with selectivity for D₂-like receptors (K_i values are 1.2, ~ 7, 2.3, ~ 80 and ~ 100 nM for D₂, D₃, D₄, D₁ and D₅ receptors respectively). Subtype-selective NMDA antagonist.

Comparison with spiperone hydrochloride: 5-HT_{2A} serotonin and selective D₂-like dopamine receptor antagonist (K_i values are 0.06, 0.6, 0.08, ~ 350, ~ 3500 nM for D₂, D₃, D₄, D₁ and D₅ receptors respectively). Antipsychotic.
 - 200 μM herkinorin (abcam, ab120147)

Dissolve 1 mg of herkinorin in 10.111 ml of appropriate buffer (BRET buffer).

Note: Herkinorin is selective μ opioid receptor agonist derived from the plant product, salvinorin A and does not promote the recruitment of β-arrestin 2 or lead to receptor internalization. Wherever possible, you should prepare and use solutions on the same day.
 - 50 mg/ml Hygromycin B (Invitrogen, 10687-010)

Hygromycin B is an aminoglycosidic antibiotic that inhibits protein synthesis by disrupting translocation and promoting mistranslation at the 80S ribosome. **Because it uses a different mode of action than Geneticin, Blastidicin S, or Zeocin, it's perfect for dual-selection experiments when used in conjunction with another selection agent.** Resistance to Hygromycin B is conferred by the E. coli hygromycin resistance gene (hyg or hph). The concentration for selection ranges from 100-1000 μg/ml (typically 200 μg/ml) and should be optimized for each cell line.
 - 3N HCl (Sigma, 320331)

Mix 2.575 ml of 37% HCl and 7.425 ml of H₂O. Stored at room temperature.

The molarity of the concentrated HCl (36.5-38%): Mass of 1 L is 1.2 g/ml x 1000 mL = 1180 g. 37% of that is HCl. 1 L of 37% HCl includes 1180 x 0.36 = 424.8 g. Number of moles is 424.8/36.46 = 11.65. The molarity of concentrated HCl is 11.65.

Note: Concentration is 37% (w/w). Density is 1.2 g/ml. Molecular weight is 36.46.
 - 1 M HEPES pH 8.0 at 4°C
Dissolve 238.3 g of HEPES, Free Acid, ULTROL Grade (CALBIOCHEM, 391338) in 600 ml of H₂O. The solution is buffered to pH 8.0 with 10 N NaOH (approximately 25 ml) at 4°C. Add distilled H₂O to 1 liter and stored at 4°C.
 - 100 mM idazoxan hydrochloride (Tocris, 0793)

Dissolve 10 mg of idazoxan hydrochloride in 415 μ l of H₂O or appropriate buffer. Store at -20°C.

· 2 M imidazole pH 8.0 at 4°C

To 34.05 g imidazole, add distilled water to 200 ml and dissolve completely. Adjust to pH 8.0 with HCl. Add distilled water to 250 ml. Use high purity imidazole as this will give no or very low absorbance at 280 nm (imidazole, 68.08 g/mol). Solutions can be successfully sterilized by autoclaving, and are stable for at least 2 years at 2-8°C, protected from light.

Note: a highly pure, low-absorbance imidazole (Fisher, BP 305-50)

· 200 mg/ml (20% w/v, 0.8M) IPTG (Research Products International, I56000)

IPTG is isopropylthio- β -D-galactoside. Make a 20% solution of IPTG by dissolving 2 g of IPTG in 8 ml of distilled H₂O. Adjust the volume of the solution to 10 ml with H₂O and sterilize the solution by passing it through a 0.22- μ m disposable filter. Dispense the solution into 1-ml aliquots and store them at -20°C.

· 3-Isobutyl-1-methylxanthine (IBMX) (Calbiochem, 410957)

· 1 M KCl

Dissolve 3.7275 g of KCl in 30 ml of H₂O and add distilled H₂O to 50 ml. Store at room temperature.

· LB (BD, 244620)

Dissolve 20 g of the powder in 800 ml of H₂O. Mix thoroughly. Autoclave at 121 °C for 20 min and cool to 25°C. Add appropriate antibiotic. Store at 4°C.

· LB agar plates

Prepare LB broth, then add agar (15 g/L), autoclave, and cool to 50°C. Add appropriate antibiotic. Pour plates and store at 4°C.

· 0.2 mM L-DOPA (Tocris, 3788)

Dissolve 197 μ g of L-DOPA and 190 μ g of sodium metabisulfite (SIGMA, 255556) in 5 ml of appropriate buffer. Sodium metabisulfite was added to prevent oxidation. Solutions must be protected from light. This solution should be prepared immediately before use.

· 100 mM LE 300 (Tocris, 1674)

Dissolve 10 mg of LE 300 in 344 μ l of DMSO and store at -20°C.

Note:

1. Potent and selective dopamine D₁ receptor antagonist (K_i values are 0.08 - 1.9 nM and 6 - 45 nM for D₁ and D₂ receptors respectively). Also displays moderate affinity for the 5-HT_{2A} receptor (K_i = 20 nM). Active *in vivo*.
2. It was not able to prepare 300 μ M LE 300 in BRET buffer.

· 10 mM MCC22 (from Philip S. Portoghese)

Dissolve 10.26 mg of MCC22 in 0.818 ml of DMSO and store at -20°C.

Note:

1. Molecular Weight: 1254

· 100 mM metoprolol tartrate

Dissolve 34.24 mg of metoprolol tartrate in 1 ml of H₂O or appropriate buffer. Store at -20°C.

Note:

1. Molecular Weight: 342.4
2. CAS Number: 56392-17-7

· β-Mercaptoethanol

0.5 M in H₂O: 260 nm = 1.5, 280 nm = 0.3

· 40 mM MG-132

To prepare x1,000 stock solution, dissolve 1.0 mg of MG-132 (TOCRIS Bioscience, 1748) in 52.6 μl of DMSO. Store at -20°C. DMSO stock solutions are stable for up to 1 month at -20°C.

· 1 M MgCl₂ (Sigma, M1028-100ML)

Dissolve 10.2 g of MgCl₂·6H₂O in 30 ml of H₂O and add distilled H₂O to 50 ml. Store at room temperature.

· 1 M MgSO₄

Dissolve 6.02 g of MgSO₄ in 30 ml of H₂O and add distilled H₂O to 50 ml. Store at room temperature.

· 50 mM morphine

Dissolve 37.94 mg of Morphine sulfate salt pentahydrate (Sigma, M8777-250MG) in 1 ml of H₂O. Store at -20°C.

Note: Do not use BRET buffer to prepare stock solution. BRET buffer cannot dissolve morphine sulfate salt pentahydrate: you may see precipitation.

· 0.9% (w/v) NaCl

Dissolve 0.45 g of NaCl in 40 ml of H₂O and adjust the volume to 50 ml with H₂O. Store at room temperature.

· 4 M NaCl

Dissolve 233.72 g of NaCl in 800 ml of H₂O and adjust the volume to 1 L with H₂O. Store at room temperature. Dissolve NaCl in DEPC-H₂O for RNase-free solution.

· 5 M NaCl

Dissolve 146.1 g of NaCl in 400 ml of H₂O and adjust the volume to 500 ml with H₂O. Store at room temperature. Dissolve NaCl in DEPC-H₂O for RNase-free solution.

· 2.73 mM naloxonazine (Tocris, 0591)

Dissolve 10 mg of naloxonazine dihydrochloride in 4.996 ml of H₂O or appropriate buffer.

Note:

1. Potent opioid antagonist, selective for μ_1 receptors.
2. Soluble to 2 mg/ml in water
3. Off-white to light tan
4. Molecular Weight: 732.7

· 3 mM naloxone hydrochloride (Tocris, 0599)

Dissolve 100 mg of naloxone hydrochloride in 2.748 ml of H₂O.

Note:

1. Competitive antagonist for μ , κ , δ , and σ opioid receptors; blocks the action of σ -agonists at opioid sites.
2. Molecular Weight: 363.84
3. Cannot prepare 100 mM stock solution with BRET buffer.

· 3 mM naltrexone (SIGMA, N3136-100MG)

Dissolve 11.3 mg of naltrexone hydrochloride in 10 ml of H₂O or appropriate buffer.

· 100 mM naltrindole (Tocris, 0740)

Dissolve 10 mg of naltrindole hydrochloride in 207.3 μ l of DMSO. Store at -20°C.

Note:

1. Highly selective non-peptide δ opioid antagonist, showing 223- and 346-fold greater activity at δ than at μ and κ opioid receptors.
2. Soluble to 10 mM in ethanol and to 25 mM in water and to 100 mM in DMSO
3. Molecular Weight: 482.48

· 10 N NaOH (for pH adjustment)

Dissolve 20 g of NaOH in 40 ml of H₂O and adjust the volume to 50 ml with H₂O. Store at room temperature.

Note:

1. Molecular Weight: 39.9971
2. CAS Number: 1310-73-2

· 0.2 mM N-formyl-Met-Leu-Phe (Tocris, 1921)

Dissolve 1 mg N-formyl-Met-Leu-Phe in 11.4273 ml of appropriate buffer. Store at -20°C.

- 50 mM norbinaltorphimine (Tocris, 0347)

Dissolve 10 mg of naltrindole hydrochloride in 272.2 μ l of DMSO. Store at -20°C.

Note:

1. Alternative Names: *nor*-BNI, *nor*-Binaltorphimine dihydrochloride
2. A selective κ -opioid receptor antagonist.
3. Soluble to 25 mM in water with gentle warming and to 25 mM in DMSO
4. Molecular Weight: 734.72

- 100 mM (-)-norepinephrine (SIGMA, A7257-500MG)

Dissolve 1.6918 mg of (-)-norepinephrine (100 mM) in 0.9 ml of H₂O or appropriate buffer and add HCl to a final concentration of 0.1 N HCl. Add H₂O to 1 ml. Store at -20°C.

- 10% (w/v) NP-40 Alternative (CALBIOCHEM, 492016)

Take 5 g of NP-40 Alternative into a 50-ml tube, add H₂O to 50 ml, and shake at room temperature until dissolve. Store at room temperature.

Note: Average molecular weight of NP-40 Alternative is 680. CMC is 50-300 μ M (0.0034-0.0204%).

- 25% (w/v) *n*-Octyl- β -D-glucopyranoside (CALBIOCHEM, 494459)

Dissolve 1.25 g of *n*-Octyl- β -D-glucopyranoside in 3 ml of H₂O and add distilled H₂O to 5 ml. Store at 4°C.

- 5% (w/v) *n*-Dodecanoylsucrose (CALBIOCHEM, 324374)

Dissolve 2.5 g of *n*-Dodecanoylsucrose (DDS) in 30 ml of H₂O and add distilled H₂O to 50 ml. Store at 4°C.

- 0.2 mM Orexin A (abcam, ab120212)

Dissolve 1 mg of Orexin A in 1.404 ml of appropriate buffer. **Store at -80°C.**

- 0.2 mM Orexin B (abcam, ab141330)

Dissolve 1 mg of Orexin B in 1.7245 ml of appropriate buffer. **Store at -80°C.**

- 4% Paraformaldehyde in PBS

Mix 12.5 ml of 16% paraformaldehyde (Electron Microscopy Science, 15710), 5 ml of 10x PBS, and 32.5 ml of H₂O. Frozen in aliquots for at least six month at -20°C. Once thawed, the aliquots can be stored at 4°C for up to one week.

- PBST

<u>Substances</u>	<u>Final conc.</u>	<u>(/20 L)</u>
10 x PBS	1 x	2 L
10% Tween 20	0.1%	200 ml

H₂O

N/A

up to 20 L

· 100 mM Pilocarpine hydrochloride (Tocris, 0694)

Dissolve 24.472 mg of pilocarpine hydrochloride in 1.0 ml of appropriate buffer (BRET buffer). Store at -20°C.

· 50 mM PMSF

Dissolve 435.5 mg of PMSF (phenylmethanesulfonyl fluoride) (SIGMA, P7626) with 50 ml of isopropanol (2-propanol) in a 50-ml tube and store at 4°C.

· 100 mM Pyrrole-2-carboxylic acid (SIGMA, P73609-1G)

Dissolve 11.11 mg of Pyrrole-2-carboxylic acid in 1.0 ml of appropriate buffer (BRET buffer) with gentle warming in a 45-60°C water bath. Store at -20°C.

· 100 mM salbutamol hemisulfate (Tocris, 0634)

Dissolve 28.8 mg of salbutamol hemisulfate in 1.0 ml of appropriate buffer or H₂O. Store at -20°C.

· 10 mM SB 2242289

Dissolve 5.9312 mg of SB 2242289 hydrochloride (TOCRIS, 1221) with 1 ml of DMSO and store at -20°C.

Note:

1. Selective 5-HT_{1B} receptor antagonist (pK_i = 8.2). Displays > 60-fold selectivity over 5-HT_{1D}, 5-HT_{1A}, 5-HT_{1E}, 5-HT_{1F}, 5-HT_{2A} and 5-HT_{2C} receptors in radioligand binding and functional assays. Centrally active following oral administration *in vivo*.

· 100 mM SB 334867 (Tocris, 1960)

Dissolve 10 mg of SB 334867 in 0.313 ml of DMSO. Store at -20°C.

Note:

Selective non-peptide orexin OX₁ receptor antagonist. pK_b values are 7.2 and < 5 for inhibition of intracellular Ca²⁺ release in CHO cells expressing human OX₁ and OX₂ receptors respectively. Blocks orexin-A induced grooming and feeding following systemic administration *in vivo*. **Could not prepare 30 μM SB 334867 with BRET buffer.**

· 100 mM SCH 39166 hydrobromide (Tocris, 2299)

Dissolve 10 mg of SCH 39166 hydrobromide in 253 μl of DMSO and store at -20°C.

Note:

1. High affinity dopamine D₁/D₅ receptor antagonist; displays K_i values of 1.2, 2, 980, 5520, 80 and 731 nM for binding to D₁, D₅, D₂, D₄, 5-HT and α_{2a} receptors, respectively.

· 100 mM SCH 442416 (Tocris, 2463)

Dissolve 10 mg of SCH 442416 in 257 μ l of DMSO and store at -20°C.

Note:

Selective adenosine A_{2A} receptor antagonist; binds to human and rat A_{2A} receptors with high affinity (K_i values are 0.048 and 0.5 nM respectively). Displays > 23000-fold selectivity for hA_{2A} over hA₁ *in vitro* with minimal affinity for hA_{2B} and hA₃ receptors (IC_{50} > 10 μ M). **Could not prepare 300 μ M SCH 442416 with BRET buffer.**

· 100 mM SCH 58261 (Tocris, 2270)

Dissolve 10 mg of SCH 58261 in 290 μ l of DMSO and store at -20°C.

Note:

1. Potent and selective A_{2A} adenosine receptor competitive antagonist (K_i = 1.3 nM). Displays 323-, 53- and 100-fold selectivity over A₁, A_{2B} and A₃ receptors, respectively. **Could not prepare 300 μ M SCH 58261 with BRET buffer.**

· 50 mM SCH 79797 dihydrochloride

Dissolve 10 mg of SCH 79797 dihydrochloride (TOCRIS, 1592) with 0.45 ml of DMSO and store at -20°C.

Note:

1. Potent, selective non-peptide PAR₁ receptor antagonist (IC_{50} = 70 nM). Inhibits haTRAP-induced- but not γ -thrombin-, ADP- or collagen-induced human platelet aggregation. Also selectively blocks PAR₁ antagonist- or thrombin-induced increases in cytosolic Ca²⁺ in vascular smooth muscle cells.

· 10x SDS running buffer for SDS-PAGE

<u>Substances</u>	<u>Final conc.</u>	<u>(/4 L)</u>
Tris-base	0.25 M	120 g
Glycine	1.91 M	576 g
SDS	1% (w/v)	40 g
H ₂ O	N/A	up to 4 L

Dissolve reagents and adjust pH to 8.3 with HCl. Add H₂O to 4 L.

· 4x SDS sample buffer

<u>Substances</u>	<u>Final conc.</u>	<u>(/50 ml)</u>
0.5 M Tris-HCl (pH 6.8)	200 mM	20 ml
SDS	4%	2 g
14.3 M β -mercaptoethanol	572 mM	2 ml
Bromophenol blue	0.32 mg/ml	16 mg
Glycerol	40%	20 ml

Dissolve reagents and add distilled H₂O to 50 ml. Prepare 1 ml aliquots and store at -20°C.

· 2 x SDS sample buffer containing 8M urea

Substances	Final conc.	Quantity (/50 ml)
Urea	8 M	24 g
0.5 M Tris-HCl (pH 6.8)	125 mM	12.5 ml
SDS	4%	2 g
2-Mercaptoethanol	10%	5 ml
Glycerol	20%	10 ml
Bromophenol blue	0.00016%	8 mg

Add H₂O to 50 ml and mix for ~2 h.

Note: Urea makes smear bands in Western blotting.

Urea (Sigma, U0631-1KG), glycerol (Sigma G7893-2L), SDS (Sigma, L3771-500G), bromophenol blue (Sigma, B8026-25G), 2-mercaptoethanol (Sigma, M3148)

· 4x SDS sample buffer with DTT

Substances	Final conc.	(/50 ml)
0.5 M Tris-HCl (pH 6.8)	200 mM	20 ml
SDS	8%	2 g
1 M Dithiothreitol (DTT)	400 mM	3.085 g
Bromophenol blue	0.32 mg/ml	16 mg
Glycerol	40%	20 ml

Dissolve reagents and add distilled H₂O to 50 ml. Prepare 1 ml aliquots and store at -80°C (up to 6 months).

· 0.1 M Serotonin hydrochloride

Dissolve 21.3 mg of Serotonin hydrochloride (100 mM) and 19.0 mg of sodium metabisulfite (100 mM) in 1 ml of appropriate buffer (BRET buffer). Store at -80°C.

Note:

1. Molecular Weight: 212.68
2. In an *in vitro* test system at pH 7.4, it was found that degradation of serotonin by ceruloplasmin was markedly inhibited by sodium metabisulfite, ascorbic acid (Chris J. D. Zarafonitis et. al., (1960) *Experimental Biology and Medicine* 105, 560-563).

· SKF 83566 hydrobromide (Tocris, 1586)

I could not prepare 3 mM SKF 83566 hydrobromide in BRET buffer.

· 96.79 mM SNC 162

Dissolve 10 mg of SNC 162 in 230.4 µl of appropriate buffer (BRET buffer) plus 15.8 µl of 3N HCl. Store at -20°C.

Note:

1. Molecular Weight: 419.61
2. Potent and selective non-peptide δ-opioid receptor agonist ($K_i = 0.63$ nM). Displays > 8000-fold

selectivity over μ -opioid receptors and is centrally active following systemic administration *in vivo*.

· 0.2 M sodium bicarbonate (NaHCO_3)

Weight out 0.84 g of sodium bicarbonate in 30 ml of water. Adjust the volume to 50 ml with H_2O . Store at room temperature.

· 0.2 M sodium carbonate (Na_2CO_3)

Weight out 10.599 g of sodium carbonate anhydrous in 400 ml of water. Adjust the volume to 500 ml with H_2O . Store at room temperature.

· 0.2 M sodium carbonate/bicarbonate buffer

Mix x ml of 0.2 M Na_2CO_3 and y ml of NaHCO_3 .

pH			
20°C	37°C	x ml 0.2 M Na_2CO_3	y ml 0.2 M NaHCO_3
9.2	8.8	10	90
9.4	9.1	20	80
9.5	9.4	30	70
9.8	9.5	40	60
9.9	9.7	50	50
10.1	9.9	60	40
10.3	10.1	70	30
10.5	10.3	80	20
10.8	10.6	90	10

· 10% (w/v) sodium deoxycholate (Sigma, D6750-100G)

Dissolve 5 g of sodium deoxycholate in 30 ml of H_2O and add distilled H_2O to 50 ml. Store at 4°C.

· 1 M sodium phosphate dibasic (Na_2HPO_4)

Dissolve 7.098 g of Na_2HPO_4 in 30 ml of H_2O and add distilled H_2O to 50 ml. Store at room temperature.

· 50 mg/ml (500x) spectinomycin dihydrochloride (Sigma, S4014)

Weight out 2 g of spectinomycin and resuspend the spectinomycin in 40 ml of water. Filter-sterilize and store at -20°C.

· 50 mM SR 27897 (Tocris, 2190)

Dissolve 10.0 mg of SR 27897 in 0.486 ml of DMSO. Store at -20°C.

Note:

1. Potent, non-peptide CCK_1 receptor antagonist that displays > 33-fold selectivity over CCK_2 receptors (EC_{50} values are 6 and 200 nM respectively). Causes an increase in plasma leptin levels and increases

food intake in rats *in vivo*.

2. To prepare 300 μ M working solution, warm the solution up to 60°C for 5 min.
3. This solution might deactivate NanoLuc.

· 0.2 mM substance P (Abcam, ab120170)

Dissolve 5 mg of substance P in 18.5515 ml of appropriate buffer. Sonicate briefly, if necessary, to dissolve, and avoid heating. Store at -20°C.

· 50x TAE

<u>Substances</u>	<u>Final conc.</u>	<u>(/2 L)</u>
Tris-base	2 M	484 g
Acetic acid, glacial	2.5 M	114 ml
0.5 M EDTA (pH 8.0)	2.0%	200 ml
H ₂ O	N/A	up to 2 L

Note: Tris-base (Fisher Scientific, BP152-1)

· 10 mM TBPB (Sigma, SML0453-5MG)

Dissolve 5 mg of TBPB in 1.236 ml of DMSO at 45°C. Store at -20°C.

Note: I could not dissolve 5 mg of TBPB (0.2 mM) in 24.719 ml of BRET buffer.

· 100 mM TC-G 1004 (Tocris, 4407)

Dissolve 10 mg of TC-G 1004 in 1.0 ml of 1N HCl. Store at -20°C. To prepare 300 μ M working solution, warm the solution up to 60°C.

· 10x transfer buffer for Western blotting

<u>Substances</u>	<u>Final conc.</u>	<u>(/2 L)</u>
Tris-base	250 mM	60.57 g
Glycine	1.92 mM	285.27 g

Dissolve reagents and add distilled H₂O to 2 L. If the pH is below 8.0, remake the buffer.

Note: Tris-base (Fisher Scientific, BP152-1), Glycine (BP381-500)

· 1x transfer buffer for Western blotting

<u>Substances</u>	<u>Final conc.</u>	<u>(/10 L)</u>
H ₂ O	N/A	7.5 L
10x transfer buffer	1x	1.0 L
Methanol	15%	1.5 L

· 6.67 mM TRAP-6 (Tocris, 3497)

Dissolve 5 mg of TRAP-6 in 1 ml of BRET buffer. Store at -20°C. You may dissolve more TRAP-6 in 1 ml

of BRET buffer.

- 0.5 M Tris-HCl pH 6.8 at room temperature

Dissolve 60.57 g of Trizma base (Sigma, T6791) in 600 ml of H₂O. The solution is buffered to pH 6.8 with HCl at room temperature. Add distilled H₂O to 1 liter and stored at room temperature.

- 10% Triton X-100 (Fluka, 93426)

Take 10 g of Triton X-100 into a 100-ml beaker containing 60 ml of H₂O and a stirrer bar, and then mix by a magnetic stirrer at room temperature until dissolve. Adjust the volume to 100 ml with H₂O. Store at room temperature.

- 100 mM tulobuterol hydrochloride (Alfa Aesar, J61448)

Dissolve 25 mg of tulobuterol chloride in 0.946 ml of appropriate buffer (BRET buffer). Store at -20°C.

- 10% Tween 20

Take ~800 ml of H₂O and 100 g of Tween 20 (SIGMA, P7949-500) into a 1-L beaker and mix the solution using a magnetic stirrer. Adjust the volume to 1 L with H₂O. Store at room temperature.

- 0.2 mM [Arg⁸]-Vasopressin (Tocris, 2935)

Dissolve 1 mg of [Arg⁸]-Vasopressin in 4.6116 ml of appropriate buffer (BRET buffer). Store at -20°C.

- 27.5 mM VU0029767 (Sigma, V3265-25MG)

Dissolve 25 mg of VU0029767 in 2.5 ml of DMSO. Store at -20°C.

- 75 mM WIN 64338 (Tocris, 1057)

Dissolve 10 mg of WIN 64338 hydrochloride with 170.1 µl of DMSO and store at -20°C.

Note:

1. bradykinin B2 R: K_i= 60 nM (human); Muscarinic acetylcholine receptor: K_i= 350 nM (rat)

- 10 mM ZnCl₂

Dissolve 68.2 mg of ZnCl₂ in 50 ml of H₂O. Store at room temperature.

Useful Data

- The average molecular weight of an amino acid is 110Da.