Screen Quest™ No Wash Potassium Channel Assay Kit

Catalog number: 36550, 36551, 36552
Unit size: 1 Plate, 10 Plates, 100 Plates

Component	Storage	Amount		
		Cat No. 36550	Cat No. 36551	Cat No. 36552
Component A: Tl-520 AM	Freeze (<-15 °C), Minimize light exposure	1 vial	1 vial	10 vials
Component B: 10X Pluronic® F127 Plus	Freeze (<-15 °C), Minimize light exposure	1 bottle (1 mL)	1 bottle (10 mL)	1 bottle (100 mL)
Component C: HHBS (Hanks with 20 mM Hepes)	Freeze (<-15 °C)	1 bottle (9 mL)	1 bottle (100 mL)	Not Included
Component D: 5X Chloride-free Assay Buffer	Refrigerate (2-8 °C)	1 bottle (2 mL)	1 bottle (20 mL)	2 bottles (100 mL/bottle)
Component E: K2SO4 (250 mM)	Refrigerate (2-8 °C)	1 bottle (2 mL)	1 bottle (2 mL)	2 bottles (100 mL/bottle)
Component F: Tl2SO4	Refrigerate (2-8 °C)	Not Provided (Sigma Cat#: 208191)	Not Provided (Sigma Cat#: 208191)	Not Provided (Sigma Cat#: 208191)

OVERVIEW

Potassium (K+) ion channel plays an important role in regulating fundamental biological processes including heart rate, hormone and neurotransmitter secretion, water and electrolyte balance. Potassium ion channel has been considered as drug targets for disease indications including arrhythmia, pain, diabetes, neurological dysfunctions etc. The permeability of Tl+ through K+ channel has been widely used to assay K+ channel. The cells that express K+ channel of interests (e.g. hERG, Kv1.3, Kir2.1, KATP) are pre-loaded with a Tl+ sensitive dye. The dye is non-fluorescent and is permeable to cell membrane. Once inside the cell, the non-fluorescent AM ester dye is cleaved by endogenous esterase into a negatively charges dye that stays inside cells. When a stimulus buffer containing low dose of Tl+ is added to cells, the Tl+ flows across the K+ channel and binds to Tl+ sensitive dye, generating a fluorescent signal. This signal is proportional to the activity of K+ channel. If an antagonist or antagonist is added to the cells, the fluorescent signal decreases or increases respectively, to reflect the inhibited or stimulated activity of K+ channel.

AT A GLANCE

Protocol summary

1. Prepare cells in growth medium
2. Add Tl-520 AM dye-loading solution (100 µL/well for 96-well plate or 25 µL/well for 384-well plate)
3. Incubate at 37°C for 1 hour
4. Add agonist/antagonist and incubate at 37 °C for 30 minutes
5. Add Tl_2SO_4/K_2SO_4 Stimulus solution (50 µL/well for 96-well plate or 12.5 µL/well for 384-well plate)
6. Monitor fluorescence intensity at Ex/Em = 490/525 nm

Important Do not add additional probenecid. Thaw all the kit components at room temperature before use.

KEY PARAMETERS

Instrument:	Fluorescence microplate reader
Excitation:	490 nm
Emission:	525 nm
Cutoff:	515 nm
Recommended plate:	Black wall/clear bottom
Instrument specification(s):	Bottom read mode/Programmable liquid handling

Other Instruments: FLIPR, NOVOStar, FlexStation, ViewLux, IN Cell Analyzer, ArrayScan, FDSS

PREPARATION OF STOCK SOLUTIONS

Unless otherwise noted, all unused stock solutions should be divided into single-use aliquots and stored at -20 °C after preparation. Avoid repeated freeze-thaw cycles.

1. Tl-520 AM stock solution:
Add 20 µL (**Cat. # 36550**) or 200 µL of (**Cat. # 36551 and # 36552**) of DMSO into the vial of Tl-520 AM (Component A), and mix them well.

Note The unused Tl-520 AM stock solution can be aliquoted and stored at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

2. Assay Buffer (1X):
Add 1 mL of 10X Pluronic® F127 Plus (Component B) into 9 mL of HHBS buffer (Component C) and mix well.

Note 10 mL of 1X assay buffer is enough for one plate. Aliquot and store unused 10X Pluronic® F127 Plus (Component B) at < -20 °C. Protect from light and avoid repeated freeze-thaw cycles.

3. Chloride Free Buffer (1X):
Add 2 mL of Chloride Free Buffer (5X) (Component D) in 8 mL of ddH_2O, and mix them well.

4. Tl_2SO_4 solution (Not provided, Sigma Cat# 208191):
Dissolve Tl_2SO_4 in ultrapure H_2O to final concentration of 80 mM.

Note Tl_2SO_4 is toxic. Take necessary precautions to prevent inhalation and skin contact.

PREPARATION OF WORKING SOLUTION

1. Tl-520 AM dye-loading solution:
Add 20 µL of Tl-520 AM stock solution (500X) into 10 mL of 1X Assay Buffer and mix well.

2. Stimulus Solution(5X):
Dilute ligands (for non-voltage gated potassium channels) or K_2SO4 (for voltage gated potassium channels) with Tl_2SO4 in 1X Chloride-free buffer.

Table 1. Reference (for voltage gated hERG channel in HEK293-$KCNH_2$ cells), concentration of Tl_2SO_4 and K_2SO_4 (for voltage gated potassium channels) or ligands (for non-voltage gated potassium channels) used for the assay should be optimized for each target channel and cell type.

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Components	Volume	5X Conc	Final Conc
Chloride-free Buffer(5X)	2 mL		
K_2SO_4 (250 mM)	1 mL	25 mM	5 mM
Tl_2SO_4 (80mM)	0.5 mL	4 mM	0.8 mM
ddH2O	5.5 mL		
Total:	**10 mL**		

PREPARATION OF CELL SAMPLES

For guidelines on cell sample preparation, please visit
https://www.aatbio.com/resources/guides/cell-sample-preparation.html

SAMPLE EXPERIMENTAL PROTOCOL

1. Add 100 µL/well (96-well plate) or 25 µL/well (384-well plate) of TI-520 AM dye-loading solution into the cell plate.

2. Incubate the dye-loading plate in a cell incubator for 1 hour, and then incubate the plate at room temperature for another 15 to 30 minutes.

   Note If the assay requires 37°C, perform the experiment immediately without further room temperature incubation.

3. Prepare the K^+ channel antagonists or agonists with HHBS.

4. Incubate the plate with antagonists (for inhibitory study) in the cell incubator for 30 minutes.

5. Add 50 µL/well (for a 96-well plate) or 12.5 µL/well (for a 384-well plate) of 5X stimulus buffer with FLIPR, FDSS or Flexstation. Run the experiment with a filter set of Ex/Em = 490/525 nm. Read the plate every 1–2 seconds for 3 minutes.

EXAMPLE DATA ANALYSIS AND FIGURES

The reading (RFU (Max-Min)) obtained from the blank standard well is used as a negative control. Subtract this value from the other standards' readings to obtain the base-line corrected values. Then, plot the standards' readings to obtain a standard curve and equation. This equation can be used to calculate Astemizole samples. We recommend using the Online Four Parameter Logistics Calculator which can be found at:

https://www.aatbio.com/tools/four-parameter-logistic-4pl-curve-regression-online-calculator

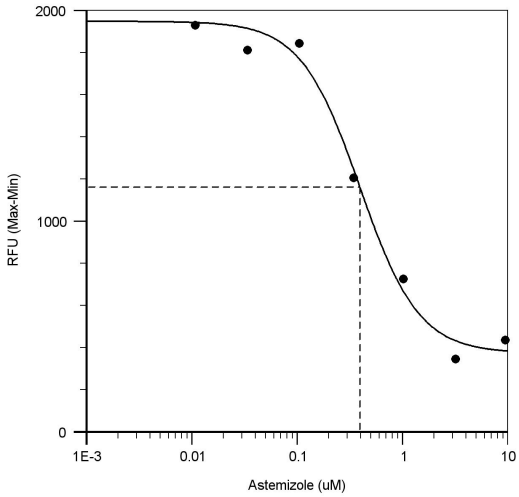

Figure 1. Astemizole dose dependent inhibition of hERG channel was measured in HEK293-KCNH2 cells with Screen Quest™ Potassium Ion Channel Kit. The cells were seeded overnight at 20,000 cells/100 µL/well in a Costar black wall/clear bottom 96-well poly-D-lysine plate. The cells were incubated with 100 µL of dye-loading solution for 1 hour at 37°C. 10 µL of Astemizole was added to the cells and incubated for 30 minutes at 37°C. 50 µL of 0.5 mM Tl2SO4 and 2.5 mM K2SO4 containing stimulus solution was injected in each well by FlexStation and read every sec for 3 minutes at excitation/emission=490/525nm. IC50 = 0.46 µM.

DISCLAIMER