CHO-Kv1.3 Cell Line

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The Kv1.3 potassium channel has shown promise as a pharmaceutical target for such diseases as multiple sclerosis, obesity, and more recently, diabetes[1]. In order to screen large compound libraries against Kv1.3, a reliable expression system is needed.

Our CHO-Kv1.3 cell line was specifically designed to express high levels of Kv1.3 channels, and to show uniform expression over time.

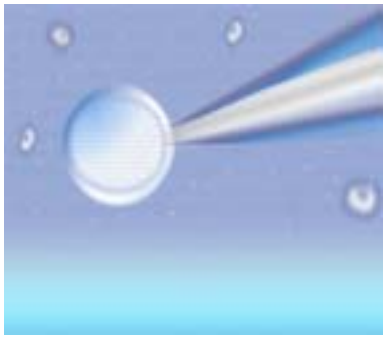
Our CHO-Kv1.3 cell line:

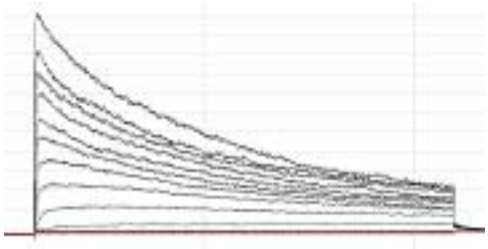
- **Stably expresses Kv1.3 potassium channels**
- **Validated using electrophysiology and efflux assay**
- **Suitable for high-throughput screening (HTS)**

Electrophysiology

Electrophysiology experiments were conducted using standard patch clamp techniques. The bath solution contained (in mM) 0.90 $CaCl_2$, 2.67 KCl, 1.47 $KHPO_4$, 0.50 $MgCl_2$, 138 NaCl, and 8.10 Na_2HPO_4. The pipette solution contained (in mM) 140 KCl, 1 $MgCl_2$, 1 EGTA, and 20 HEPES.

For I-V plots, cells were held at -80mV, and then stepped to a depolarizing voltage for 1s to record the peak current.





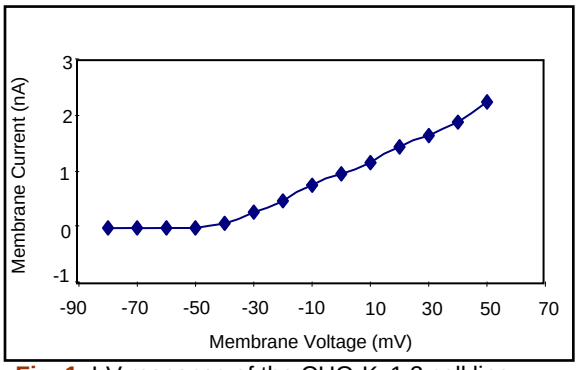


Fig. 1. I-V response of the CHO-Kv1.3 cell line.

For IC_{50} experiments, a test pulse was applied until the peak current stabilized (Fig. 2).

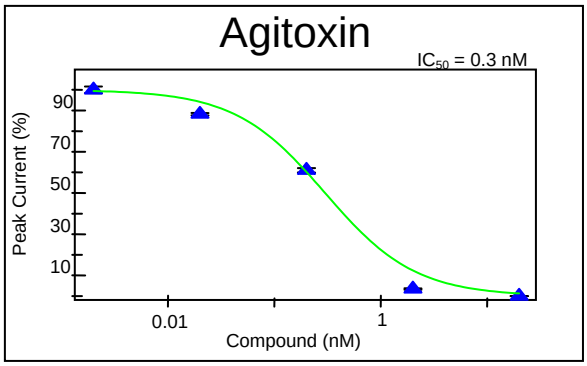


Fig 2. IC_{50} curve of Agitoxin using the CHO-Kv1.3 cell line as determined by patch clamp.

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Rubidium Efflux Assay

Using Aurora Biomed's Rubidium Efflux Assay protocol, basal efflux was measured at 8.5%. After a 6 minute activation time using 63mM KCl, the maximal activation-induced efflux was 49.3% resulting in about six fold window of detection (Fig. 3).

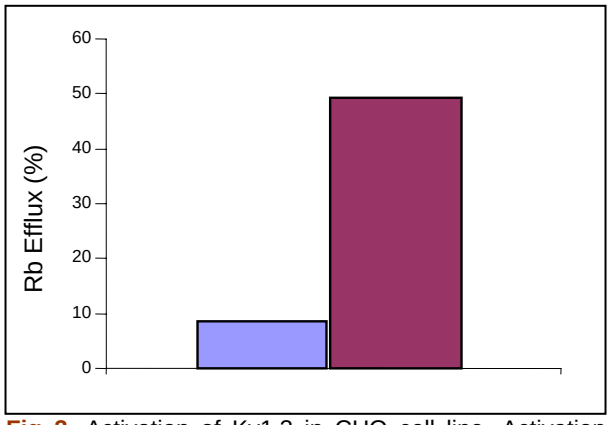

Fig 3. Activation of Kv1.3 in CHO cell line. Activation time of 6 min with 63 mM KCl leads to a window of approximately 41% from basal efflux (blue bar) to activated efflux (red bar).

The concentration-response curves of two commonly known Kv1.3 blockers, Agitoxin and Margatoxin were determined by Rubidium Efflux Assay using the CHO-Kv1.3 cell line (Fig. 4). Table 1 is included to summarize some results and information on our Kv1.3 cell line

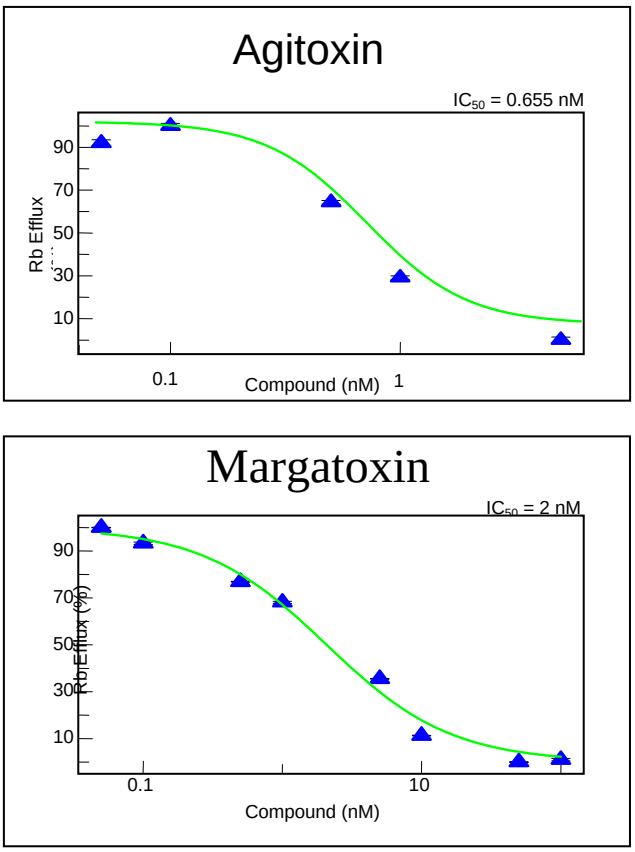

Fig. 4. IC_{50} curve of Agitoxin and Margatoxin on CHO-Kv1.3 as determined by Aurora Biomed's Rubidium Efflux Assay.

Table 1. Summary of data for CHO-Kv1.3 cell line.

Cell Line	Activation KCl (mM)	Activation period (min)	Published EP IC_{50} for Agitoxin (nM).[1]	Aurora EP IC_{50} for Agitoxin (nM).	Aurora Flux IC_{50} for Agitoxin (nM).
CHO Kv1.3	63	6	0.2	0.3	0.655

References

1. Wulff H. et al., (2003). The voltage-gated Kv1.3 (+) channel in effector memory T cells as new target for MS. *J Clin Invest*, 111(11): 1703-13.
2. Gill S. et al., (2005). Cell-based rubidium flux assay for HTS of Kv1.3 channels. *Society for Biomolecular Screening Poster Presentation.*