# Application Note

Channel: hTRPM8
Cells: CHO
Tools: Port-a-Patch

# Cold activation of TRPM8 using the External Perfusion System and the Port-a-Patch

The electrophysiology team at Nanion Technologies GmbH, Munich.

## Summary

TRPM8 is a member of the transient receptor potential channel (TRP) family. TRPM8 is known to be a thermo-sensitive channel, activated by cold temperatures (below ~25˚C) and ligands such as menthol, Eucalyptol and icilin[1-4]. It belongs to the melastatin subfamily of TRP channels[5] and shows an outward rectification with a relatively high permeability for calcium ions and little selectivity between monovalent cations. Menthol, a secondary alcohol produced by the peppermint herb, *Mentha piperita*, is widely used in the food and pharmaceutical industries as a cooling/soothing compound and odorant. It induces $Ca^{2+}$ influx in a subset of sensory neurons from dorsal root and trigeminal ganglia, where the TRPM8 channel is specifically expressed[2-4]. It is known that TRPM8 plays a role cold allodynia after inflammation[5] and it has been implicated as a possible pharmaceutical target to treat chronic pain and migraine[6]. Additionally, TRPM8 channels have been implicated to play a role in cancer[7,8], in particular prostate cancer[7] and pancreatic cancer[8], and may provide novel clinical biomarkers and therapeutic targets for these types of cancer.

Here we present data of hTRPM8 collected on the Port-a-Patch using cooled solution *via* the External Perfusion System. Cold activated hTRPM8 was blocked by capsazepine with an $IC_{50}$ in good agreement with the literature[1,9].

## Results

TRPM8 can be activated by a number of ligands including the cooling compounds menthol and icilin[1-4], as well as cold temperatures[1-4]. TRPM8 is a cation channel with voltage-dependence of activation exhibiting a strong outward rectification at depolarized membrane potentials and fast, voltage-dependent closure at negative potentials[9,10]. TRPM8 -mediated currents are suppressed by high temperatures[2,10]. Figure 1 shows activation of TRPM8 expressed in CHO cells by continuous perfusion of external solution at 10°C and subsequent suppression by elevated temperature (35°C).

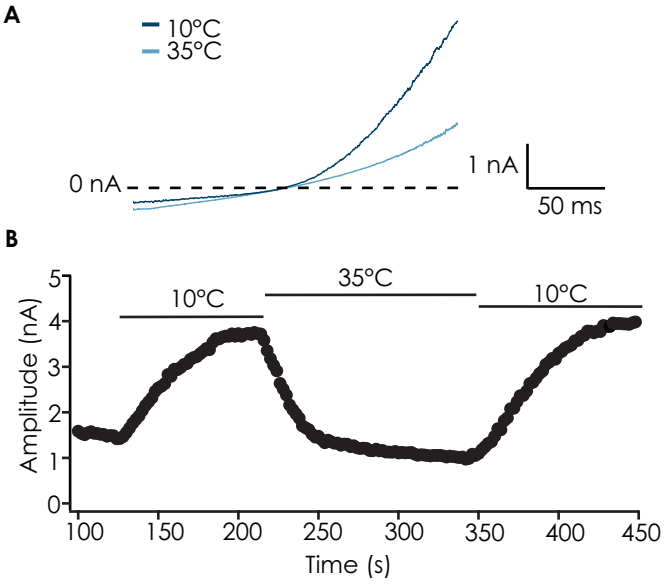


**Figure 1: A** Whole cell current responses from induced CHO cells expressing TRPM8 to a ramp protocol (-100 mV to +100 mV over 200 ms) at 10°C (dark blue) and 35°C (light blue). TRPM8 was activated at 10°C but not at 35°C. **B** Timecourse of TRPM8 activation with cold solution (10°C) and block by warm solution (35°C).

# Application Note

TRPM8 was activated by cooled solution and this could be blocked by either increasing temperature, or by applying the blocker capsazepine *via* the external solution. Capsazepine was applied at 10°C. Figure 2 shows the time course of the exeriment and corresponding example traces.

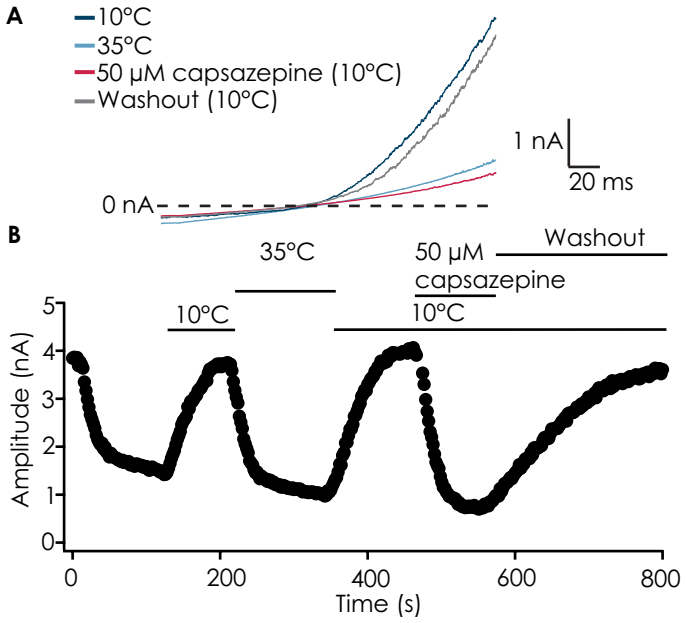


**Figure 2: A** Traces from an example cell showing activation by cooled solution (10°C, dark blue), block by either heated solution (35°C; light blue), or capsazepine (50 µM; red trace) and washout after capsazepine application (grey). TRPM8 current could be almost completely recovered upon washout of capsazepine. **B** Timecourse of the experiment.

A concentration response curve for capsazepine using cooled solution (10°C) as the activator could also be constructed. Increasing concentrations of capsazepine at 10°C were applied and the concentration response curve is shown in Figure 3. The $IC_{50}$ for capsazepine block of TRPM8 was 12.9 µM (n = 1), in excellent agreement with the literature[1,9].

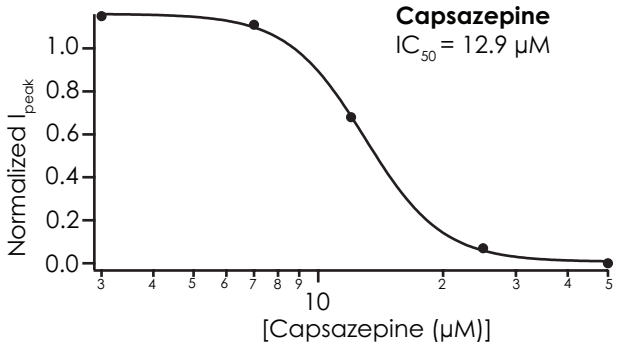


**Figure 3:** Concentration response curve for capsazepine using cooled solution to activate TRPM8 reveals an $IC_{50}$ of 12.9 µM, in good agreement with the literature[1,9].

In conclusion, hTRPM8 expressed in CHO cells can be reliably activated using the External Perfusion System of the Port-a-Patch with Temperature Control which enables cooling of the external solution to 10°C. Cold-activated TRPM8 was also blocked by capsazepine as expected[1,9]. The Port-a-Patch is therefore a useful tool for identifying TRPM8 inhibitors as potential therapeutics for chronic pain and some cancers.

## References


1. Behrendt, H.J., *et al.*, 2004. Br. J. Pharmacol. 141, 737–745
2. McKemy, D.D., *et al.*, 2002. Nature 416: 52-58
3. Peier, A.M., *et al.*, 2002. Cell 108: 705-715
4. Andersson, D.A., *et al.*, 2004. J. Neurosci. 24(23):5364–5369
5. Weyer, A.D. & Leyto, S.G. 2017. Pharmaceuticals. 10: 37
6. González-Muñiz, R., *et al*. 2019. Int. J. Mol. Sci. 20: 2618
7. Hantute-Ghesquier, A. et al. 2018. Pharmaceuticals. 11: 58
8. Yee, N.S. et al., 2012. Scientifica. 2012: Article 415158
9. Malkia, A., *et al.*, 2009. Mol. Pain. 6: 62
10. Nilius, B., et al. 2005. J. Physiol. 567.1: 35–44


## Methods

### Cells

CHO cells stably expressing hTRPM8

### Cell culture

CHO cells stably expressing hTRPM8 were cultured using standard culture conditions for the Port-a-Patch and induced using 1 mg/ml tetracycline for at least 24-36 hours prior to measurements.

### Electrophysiology

Whole cell patch clamp recordings were conducted according to Nanion's standard procedure for the Port-a-Patch. Currents were elicited by 200 ms voltage ramps from -100 mV to +100 mV, Vhold = -80 mV. External solution was cooled to 10°C using the Temperature Control of the External Perfusion System to activate hTRPM8. Capsazepine concentrations were made up in external solution and applied to the cell at 10°C using the External Perfusion System of the Port-a-Patch.


Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de


nan]i[on