

## TRPM7 Channel Is Regulated by Magnesium Nucleotides via its Kinase Domain

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### External Solution

Described in the paper.

### Internal Solution

Standard intracellular pipette-filling solutions (SIS) contained (in mM) Cs-glutamate 140, NaCl 8, Cs-BAPTA 10, HEPES·CsOH, pH 7.2 adjusted with CsOH.

### Figure 1

(A) See Fig. 1 (E and F)

(B) See Fig. 4 D

(C) 6 mM AMP

0 mM free  $Mg^{2+}$ : SIS + 6 mM NaAMP

753  $\mu M$  free  $Mg^{2+}$ : SIS + 6 mM NaAMP + 1.2 mM  $MgCl_2$

(E and F) 6 mM MgATP (and 6 mM NTP) dose–response curve

0 mM free  $Mg^{2+}$ : SIS + 6 mM NaNTP

122  $\mu M$  free  $Mg^{2+}$ : SIS + 13 mM NaNTP + 7.1 mM  $MgCl_2$

160  $\mu M$  free  $Mg^{2+}$ : SIS + 10 mM NaNTP + 6.2 mM  $MgCl_2$

211  $\mu M$  free  $Mg^{2+}$ : SIS + 12 mM NaNTP + 8.25 mM  $MgCl_2$

788  $\mu M$  free  $Mg^{2+}$ : SIS + 6.8 mM NaNTP + 7 mM  $MgCl_2$

1595  $\mu M$  free  $Mg^{2+}$ : SIS + 6.4 mM NaNTP + 8.03 mM  $MgCl_2$

3190  $\mu M$  free  $Mg^{2+}$ : SIS + 6.2 mM NaNTP + 10.03 mM  $MgCl_2$

### Figure 2

(A and B) Magnesium dose–response curve

0 mM free  $Mg^{2+}$ : SIS

211  $\mu M$  free  $Mg^{2+}$ : SIS + 271  $\mu M$   $MgCl_2$

788  $\mu M$  free  $Mg^{2+}$ : SIS + 1.008 mM  $MgCl_2$

1595  $\mu M$  free  $Mg^{2+}$ : SIS + 2.03 mM  $MgCl_2$

3190  $\mu M$  free  $Mg^{2+}$ : SIS + 4.03 mM  $MgCl_2$

(C–F) 1 mM MgATP dose–response curve

0 mM free  $Mg^{2+}$ : SIS + 1 mM NaATP

211  $\mu M$  free  $Mg^{2+}$ : SIS + 1.5 mM NaATP + 1.27 mM  $MgCl_2$

788  $\mu M$  free  $Mg^{2+}$ : SIS + 1.14 mM NaATP + 2.01 mM  $MgCl_2$

1570  $\mu M$  free  $Mg^{2+}$ : SIS + 1.07 mM NaATP + 3.03 mM  $MgCl_2$

2 mM MgATP dose–response curve

0 mM free  $Mg^{2+}$ : SIS + 2 mM NaATP

211  $\mu M$  free  $Mg^{2+}$ : SIS + 3 mM NaATP + 2.27 mM  $MgCl_2$

788  $\mu M$  free  $Mg^{2+}$ : SIS + 2.2 mM NaATP + 3.01 mM  $MgCl_2$

1570  $\mu M$  free  $Mg^{2+}$ : SIS + 2.13 mM NaATP + 4.03 mM  $MgCl_2$

4 mM MgATP dose–response curve

0 mM free  $Mg^{2+}$ : SIS + 4 mM NaATP

211  $\mu M$  free  $Mg^{2+}$ : SIS + 6 mM NaATP + 4.27 mM  $MgCl_2$

788  $\mu M$  free  $Mg^{2+}$ : SIS + 4.54 mM NaATP + 5.01 mM  $MgCl_2$

1570  $\mu M$  free  $Mg^{2+}$ : SIS + 4.27 mM NaATP + 6.03 mM  $MgCl_2$

### Figure 3

(A) HEDTA-BAPTA comparison

Standard intracellular pipette-filling solutions (SIS 2) contained (in mM) Cs-glutamate 140, NaCl 8, HEPES·CsOH 10, pH 7.2 adjusted with CsOH.

HEDTA 0  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM HEDTA

BAPTA 0  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM BAPTA

HEDTA 3190  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM HEDTA + 12.92 mM  $\text{MgCl}_2$

BAPTA 3190  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM BAPTA + 4.03 mM  $\text{MgCl}_2$

(B) Kozac et al.'s conditions

270  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 12 mM EGTA + 0.5 mM  $\text{MgCl}_2$

4 mM MgATP + 360  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 3 mM EGTA + 2.5 mM HEDTA + 5 mM NaATP + 6.5 mM  $\text{MgCl}_2$

(C) HEDTA with MgATP

0  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM HEDTA + 7.25 mM  $\text{MgCl}_2$

4 mM MgATP + 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 10 mM HEDTA + 6 mM NaATP + 11.25 mM  $\text{MgCl}_2$

(D) Unbuffered conditions

652  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 652  $\mu\text{M}$   $\text{MgCl}_2$

4 mM MgATP + 652  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 2** + 4.65 mM MgATP

(E) Hermosura et al.'s conditions

Standard intracellular pipette-filling solutions (SIS 3) contained (in mM) Cs-glutamate 140, NaCl 8, EGTA 10,  $\text{CaCl}_2$  2.5, HEPES·CsOH 10, pH 7.2 adjusted with CsOH.

0 mM free  $\text{Mg}^{2+}$ : **SIS 3**

367  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 3** + 0.5 mM  $\text{MgCl}_2$

737  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : **SIS 3** + 1 mM  $\text{MgCl}_2$

#### Figure 4

(A and B) MgGTP dose–response curve, see Fig. 1 (E and F)

(C) MgNTP dose–response curve, see Fig. 1 (E and F)

(D–F) 6 mM MgADP (and 6mM NDP) dose–response curve

0 mM free  $\text{Mg}^{2+}$ : SIS + 6 mM NaNDP

160  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 10 mM NaNDP + 6.2 mM  $\text{MgCl}_2$

211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 12 mM NaNDP + 8.25 mM  $\text{MgCl}_2$

784  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 6.8 mM NaNDP + 7 mM  $\text{MgCl}_2$

1570  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 6.2 mM NaNDP + 10.03 mM  $\text{MgCl}_2$

#### Figure 5

(A) Comparison between ATP-GTP-ITP at 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$

211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 271  $\mu\text{M}$   $\text{MgCl}_2$

6 mM NTP + 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 9 mM NaNTP + 6.27 mM  $\text{MgCl}_2$

(B) Comparison between ATP and ITP at 1595  $\mu\text{M}$  free  $\text{Mg}^{2+}$

1595  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 2.03 mM  $\text{MgCl}_2$

6mM NTP + 1595  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 6.4 mM NaNTP + 8.03 mM  $\text{MgCl}_2$

(C and D) Comparison between ATP-GTP-ITP at 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$  in TRPM7 mutants

See Fig. 5 A

(E) Comparison between ATP-GTP-ITP at 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$  in TRPM7  $\Delta$ -kinase mutant

211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 271  $\mu\text{M}$   $\text{MgCl}_2$

1 mM NTP + 211  $\mu\text{M}$  free  $\text{Mg}^{2+}$ : SIS + 1.5 mM NaNTP + 1.27 mM  $\text{MgCl}_2$