

Screening with MDC's Membrane Potential Kit

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Why measure membrane potential?

ion probes

radioisotopes

ligand binding

Use of voltage dye on FLIPR

KCl induced depolarization

hyperpolarization

addition artifacts

loading time

dye concentration

Conclusions

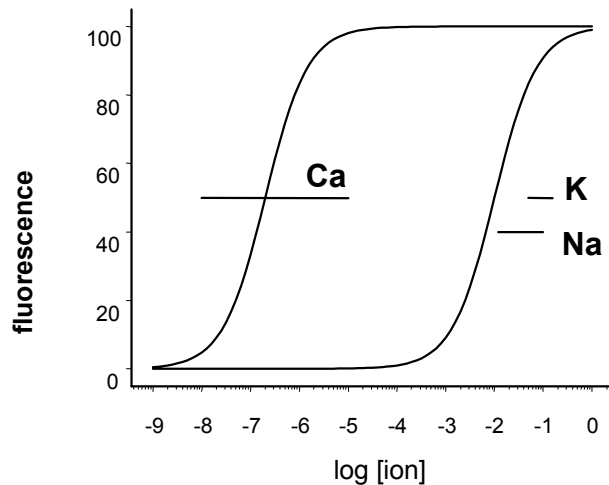
methods for assaying ion channels

electrophysiology

whole cell voltage clamp, TEVC oocytes
good kinetic and mechanistic detail
very low throughput

ion probes

calcium green, fluo 3
high throughput, good kinetic detail
not useful for most ions



radioisotope flux

any ion can be measured
high throughput
often unphysiological-membrane fractionation
poor kinetic information

ligand binding

good for ligand gated channels or GPCRs
high throughput
no kinetic information
nonfunctional assay-miss potential hits

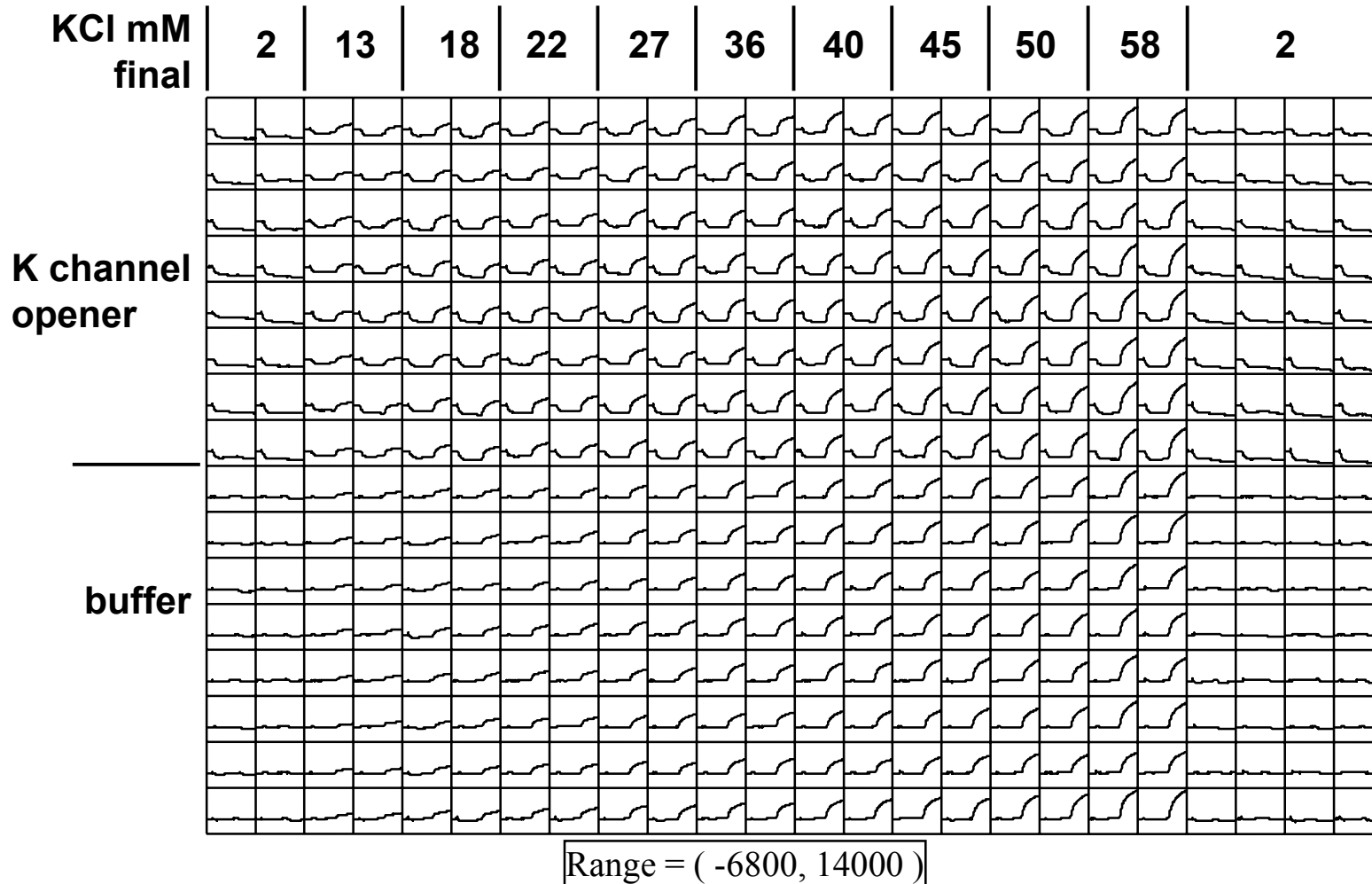
membrane potential dye

high throughput
functional assay
good kinetic detail
works with all ions
small current gives big voltage change
 $V=IR$

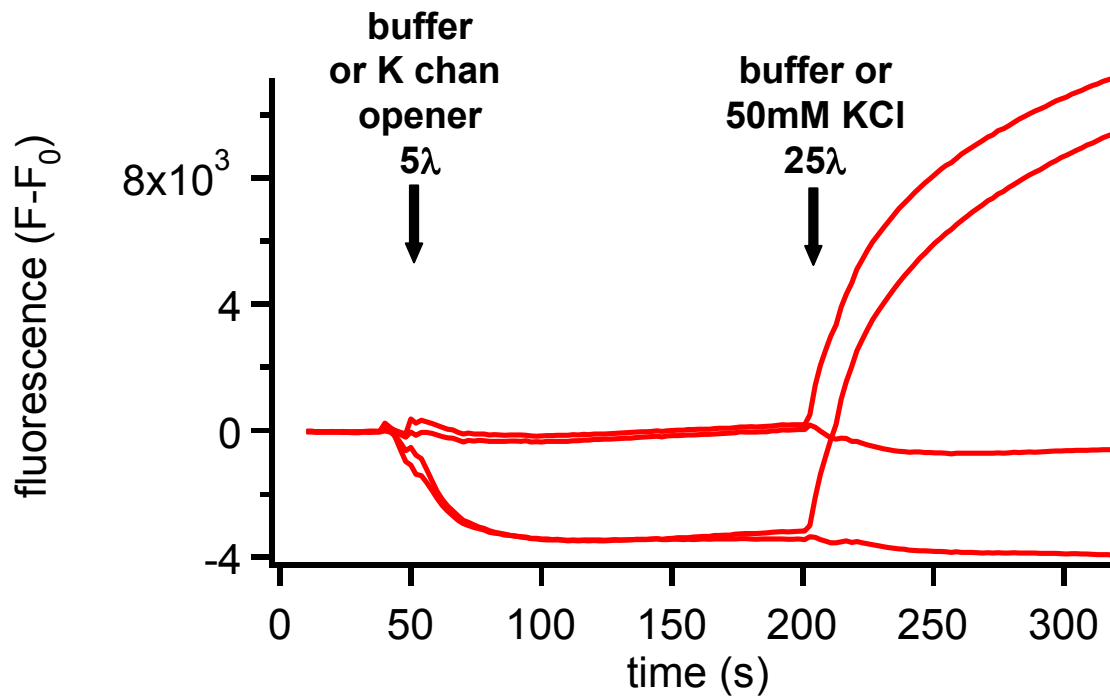
Typical procedure

- 1) Plate tsA201 cells near confluency for 24 hours
7x10⁶ cells/384 well plate using multidrop**
- 2) Remove growth medium
only necessary if constrained by volume**
- 3) Add 25 ul dye solution at recommended concentration**
- 4) Incubate with dye for 15 minutes**
- 5) Run of FLIPR with 550LP filter
works OK with standard fluo3 filter but not optimal**

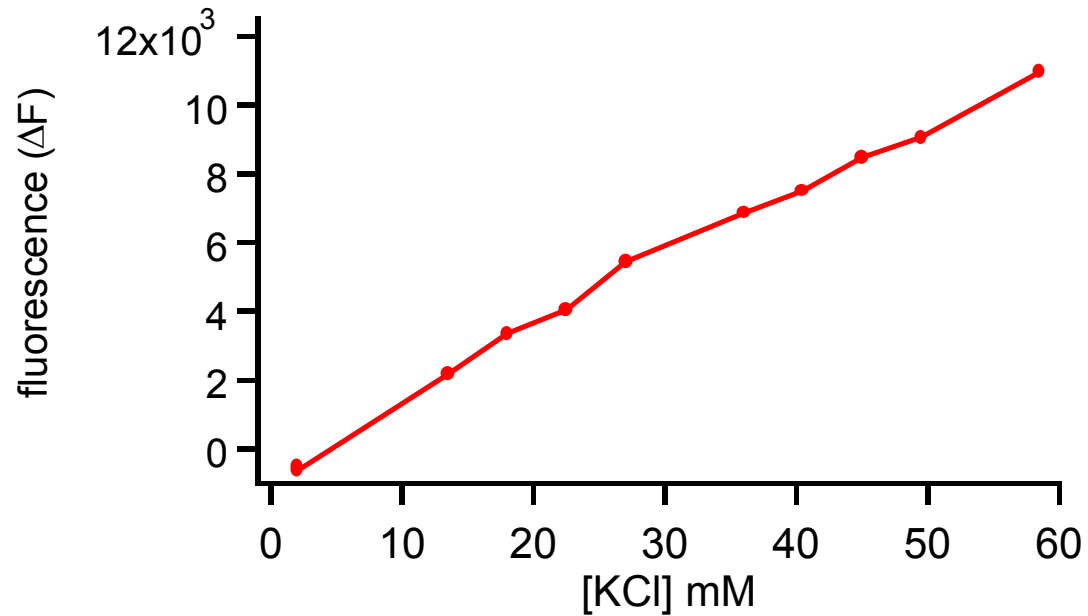
KCl induced depolarizations



Works in both directions



[KCl] dependence magnitude of the depolarizing response



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addition artifacts

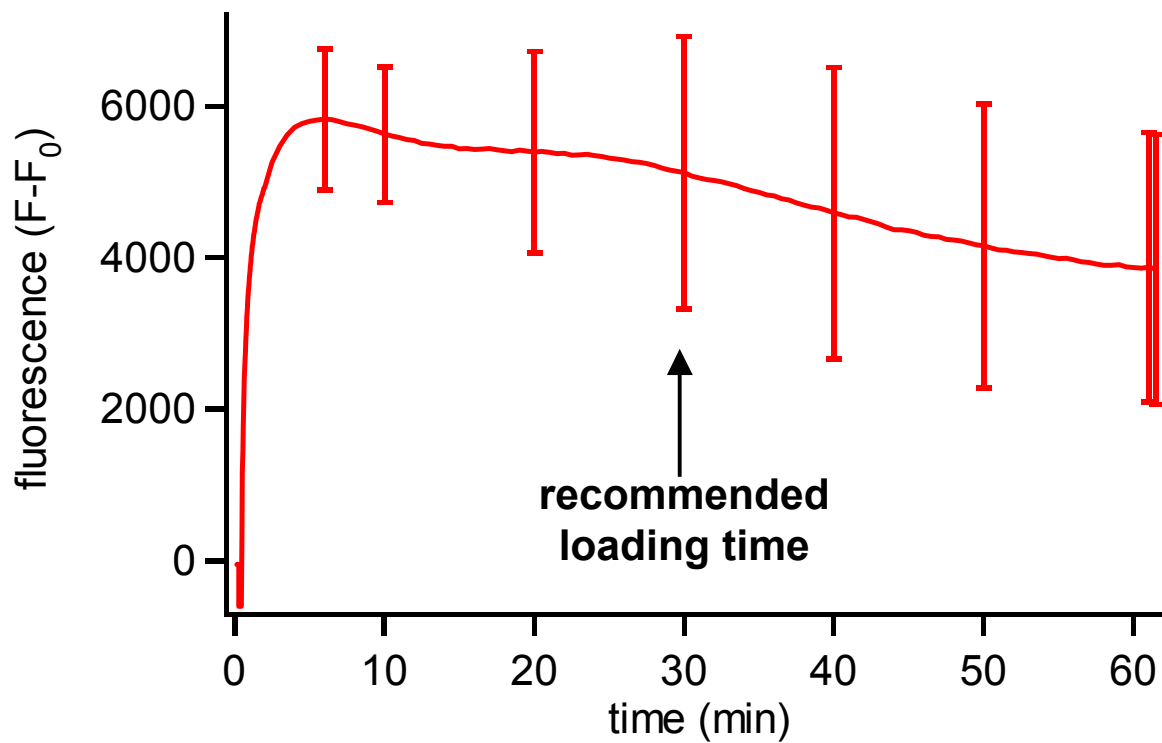
loading time

dye concentration

Conclusions

loading kinetics

online dye addition



Signal Test

X

A	11918.8	12366.9	9326.1	10737.6	11486.8	12400.8	10370.3	9916.0	10249.1	10569.4	10447.8	10994.8	9792.4	10562.9	10303.0	10191.9	9393.8	10647.3	10347.7	10954.5	10034.3	10140.7	11233.6	11289.5
B	10589.1	9917.4	8560.8	8780.5	9483.0	9379.6	8531.9	8100.6	9526.0	9210.8	9592.5	8763.0	9671.6	9105.0	9361.0	9164.7	9941.2	10244.1	11030.6	10555.9	10807.0	10895.3	11780.4	12203.8
C	11599.6	12095.2	10010.5	11182.2	11697.7	11894.8	10220.8	9879.8	9883.5	10864.6	10895.5	10157.5	10201.4	10613.9	10201.8	10287.2	10753.8	11479.6	11256.1	11299.4	11258.5	11483.3	12131.6	12262.4
D	11231.7	11494.9	8718.1	10287.4	11175.2	11888.7	9961.8	10392.6	10377.0	11225.6	10331.6	10298.4	10991.2	10507.3	11019.2	11728.2	11285.6	10942.9	12048.2	11764.8	11686.9	12564.5	12544.3	13082.4
E	10008.7	12231.1	11331.7	12094.9	11710.5	12226.5	10989.5	11534.9	11588.7	12065.9	11584.9	11381.8	12019.9	11654.9	11884.3	11411.6	11799.6	11589.8	12104.3	12237.3	12011.5	12076.1	12443.8	12213.4
F	10309.0	10365.0	8726.5	9484.0	10428.4	10997.2	9081.1	10337.1	9373.4	11105.0	9912.1	10503.8	10239.4	10764.3	9693.6	10516.1	9970.9	10780.8	11031.7	12079.3	11129.6	12354.5	12490.8	13539.2
G	11011.4	11347.0	9570.9	11553.2	10382.9	12763.1	10121.2	10343.4	11324.0	11470.8	11216.8	10604.0	11436.2	11211.3	11612.2	11439.9	11634.2	11635.4	12538.2	12887.5	11647.6	12172.5	12742.9	13361.6
H	10981.0	11764.2	9413.4	10538.5	11568.7	12507.9	10159.7	10976.1	11739.6	12262.1	11826.2	11607.8	10996.9	11168.8	10921.3	11253.9	11808.8	11556.8	12448.1	12742.9	12672.8	12847.4	13431.7	14190.3
I	11755.4	12742.7	11112.2	10944.3	12058.3	11505.3	11040.0	11830.4	11601.1	12679.0	11902.1	10716.3	11967.4	11225.8	11441.7	11919.8	11767.5	11731.5	11912.5	11985.0	13194.5	12427.8	14033.4	13383.7
J	11461.9	12377.2	10720.1	10784.1	12677.3	13008.3	10513.5	11004.6	11246.5	10764.9	11390.7	11080.5	11236.8	11008.5	11515.9	11005.9	11760.9	11766.0	13186.0	12682.9	12851.1	13160.9	13725.9	13711.8
K	13105.0	12847.7	11540.6	12407.0	13910.6	13775.6	11806.1	11822.1	12247.0	12427.5	11787.1	11431.2	11221.5	11170.9	11261.3	12385.8	11335.7	11145.6	12005.6	11207.1	12297.8	12295.6	12329.6	13631.9
L	12835.4	12830.8	10484.1	11164.3	13843.6	13358.8	11567.8	11436.7	11798.8	12888.2	11827.0	12023.2	12164.2	12053.3	11480.3	11750.1	13104.7	13027.4	13832.4	13508.1	13147.6	14585.1	14278.3	13709.4
M	12583.4	11604.1	11128.2	10926.1	12102.5	12422.9	11064.5	11548.6	11653.1	12471.7	12162.6	11905.6	11347.4	12099.4	11200.9	11821.3	11906.0	13285.5	12053.4	12038.2	12710.1	12736.3	13094.2	13667.5
N	11810.0	11140.6	10647.6	10477.3	13235.4	11983.4	11420.9	10503.8	12308.6	11787.0	11886.8	10272.1	11193.4	10800.3	11530.8	11257.1	12186.6	12373.6	12850.0	12413.3	13200.1	13410.6	13376.2	13860.1
O	12564.0	12237.7	11346.5	11306.7	12087.2	12508.0	11130.4	10925.9	11525.2	11874.8	11233.3	10464.8	11178.6	10298.7	11257.1	11061.9	10883.2	10366.8	10535.3	10829.3	10564.8	11291.0	11218.8	10760.6
P	10961.0	11827.3	10553.5	10734.3	11234.3	12797.7	10590.7	11334.2	11831.6	12174.6	11710.6	10715.3	11036.0	11463.5	10423.8	11030.0	11716.4	13076.6	12093.6	14350.6	13596.7	11998.3	13263.7	13134.3

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Average 11486.4 Minimum 8100.6 (-29.5%) Maximum 14585.1 (27.0%) Standard Deviation 1127.2 (9.8%)

Refresh

Save

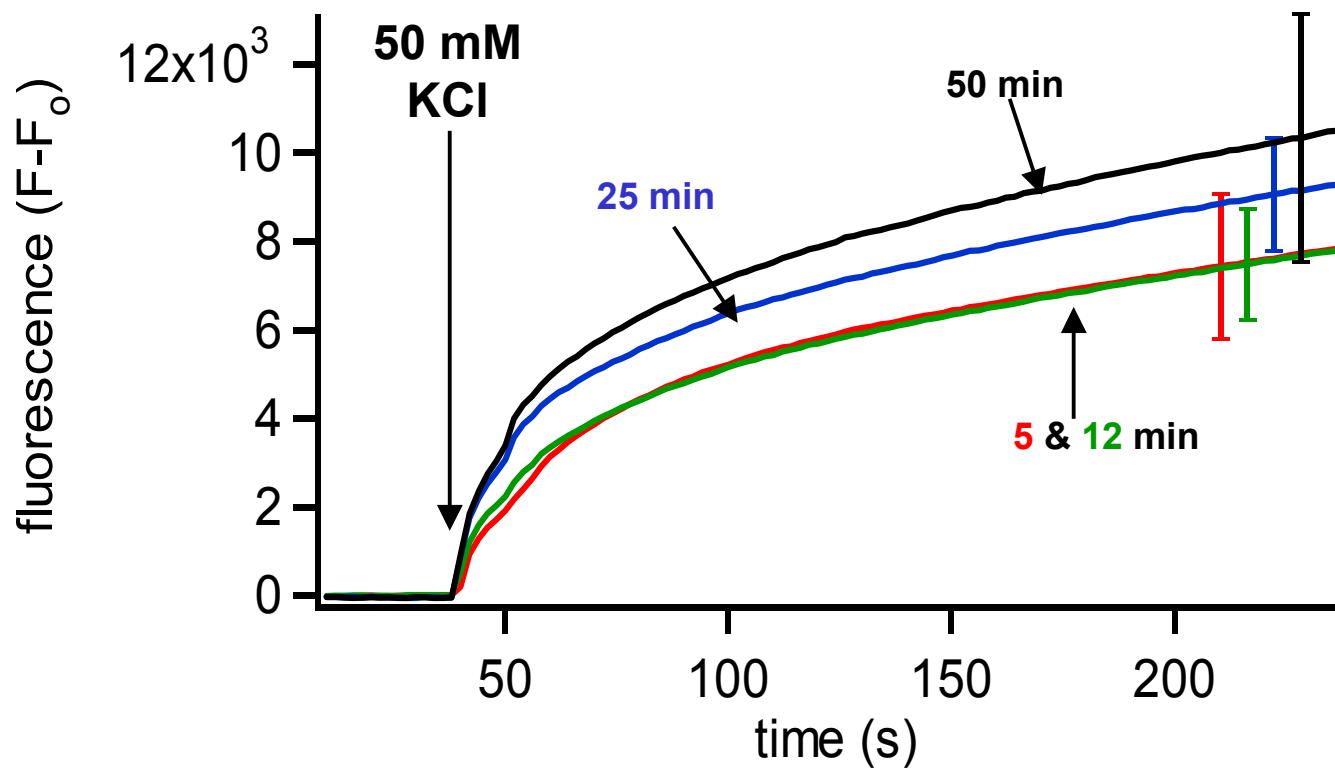
Print

Exit

20 %

Highlight Margin

signal vs loading time



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hyperpolarization

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dye concentration

Conclusions

Cost

\$25/plate

with standard procedure

1 vial = ten 96 or 384 well plates

simple ways to reduce cost

- 1) reducing total volume by aspirating growth medium
\$9/plate if 25ul total volume in 384 well
- 2) diluting dye below recommended concentration
\$12.50/plate if dye reduced by 50%

Baseline signal with varying dye concentrations

% of recommended concentration

100

75

50

25

10

100

Signal Test x

A	12312.9	12121.5	9248.9	10341.3	10384.2	10887.1	9587.4	9879.9	8932.8	9250.1	9484.3	9738.4	9862.0	10123.1	9839.9	9487.8	13823.2	12580.6	12834.8	13287.4	11032.9	9811.3	18588.6	11823.3
B	10123.9	9369.7	7853.5	9201.9	8805.3	8851.2	7721.3	7246.5	7982.3	8192.6	8258.9	7465.0	10179.7	10746.1	9906.1	8950.9	15338.4	14993.8	15216.7	15888.9	8391.6	9466.4	8023.0	10271.3
C	10452.4	11036.2	8861.9	10148.7	10095.4	11314.5	8970.8	8024.8	8822.5	9150.8	9158.8	8794.5	9940.3	10744.1	9509.3	9004.4	14188.1	15729.4	14000.7	18415.0	10210.6	9408.2	18754.3	10590.0
D	9455.9	9950.4	8781.2	9387.6	9806.1	11387.2	9244.3	8520.1	8858.8	9359.5	9662.8	8310.1	9807.9	9878.8	8341.5	7922.8	12212.7	11982.1	11414.1	11238.8	10895.9	9660.5	18532.3	12503.2
E	11330.2	10271.8	9136.6	9541.9	10297.1	9184.7	8254.9	8512.8	8570.3	9814.0	9701.8	9882.3	14801.6	17886.9	14297.5	14720.7	22413.1	22384.6	22503.4	23211.8	8657.4	8794.3	8958.9	9328.6
F	8913.4	7822.8	8441.2	9580.4	9156.8	9542.2	9810.0	8641.2	8735.0	9271.3	8817.8	8575.8	8734.8	8678.8	9130.7	8198.0	11678.5	11827.7	11927.4	13135.6	10757.4	10494.8	18795.5	11466.6
G	10999.0	10641.7	8211.3	9532.0	9065.9	10851.5	8080.9	8560.8	8888.3	9082.5	9428.8	8582.3	11978.7	12877.8	11892.2	9760.6	17447.8	17252.5	16901.1	17838.5	10133.1	10078.3	11914.0	11304.3
H	11288.4	9823.9	9148.9	9488.3	10634.8	10888.8	9142.9	8838.4	9181.4	9504.3	9236.2	8799.9	9110.2	10628.4	9421.8	8433.2	13884.1	12183.8	12412.8	13488.7	10790.8	10234.3	11785.4	12849.0
I	11722.1	11865.7	9288.9	11048.2	11223.2	10329.7	9634.0	9661.9	9515.6	9790.9	9288.5	9170.2	9529.4	8758.7	9883.6	8902.8	14044.5	12509.1	12973.5	12888.7	11480.6	10812.1	11419.7	12586.0
J	11914.0	10849.9	8882.8	9970.0	10081.5	10898.3	9559.8	8374.3	9858.9	9809.1	9825.3	9129.0	11917.7	12489.3	12132.0	10348.8	17888.8	18722.9	18168.4	18551.2	11475.6	10338.8	18872.8	11598.8
K	10401.7	11222.9	8448.5	10122.9	10513.7	11128.0	9735.8	9368.8	9437.9	9326.4	9433.3	8985.5	10987.8	12382.0	11518.8	9511.0	18548.8	17921.5	18167.5	18888.2	9587.3	9644.3	18883.8	10931.4
L	7833.3	10897.3	10883.3	10477.0	8765.7	8586.9	9889.4	9889.5	9888.8	10880.0	10373.7	9358.3	9054.2	10383.7	9217.4	8647.5	13828.8	12505.0	12891.9	12471.1	11779.9	11514.7	12838.1	13108.9
M	10309.6	11723.7	8383.1	10406.2	11160.8	10583.8	8996.5	8727.9	8885.5	9745.7	9668.8	9574.1	9320.1	9877.8	9427.1	8343.9	13532.3	12226.5	12672.8	13874.9	11179.2	11641.1	11840.0	12614.8
N	11779.5	10650.4	9885.9	9287.0	10042.8	10837.8	8473.5	9214.7	9811.8	9180.9	9481.7	8884.5	11950.2	11543.8	12297.3	9025.3	17377.3	15712.2	16736.9	18881.1	10802.6	12009.2	11824.5	12251.2
O	11477.2	11621.1	9857.9	9874.6	10166.8	10888.0	9189.3	8788.8	8935.2	9038.2	9656.8	8429.3	9557.3	10183.0	9795.5	8739.4	14478.8	15344.6	14870.8	15483.9	10883.7	9372.3	18830.5	11553.4
P	11477.9	10690.4	9886.0	9382.1	10502.8	10788.5	9177.9	8525.5	8577.7	8950.1	9114.8	8397.0	9685.2	10828.1	10116.1	8654.8	14884.2	14726.9	14800.8	15275.6	11034.0	10708.4	11279.7	12369.0

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24

Average 10806.5 Minimum 6813.4 (-37.0%) Maximum 23211.8 (114.8%) Standard Deviation 2547.5 (23.6%)

Highlite Margin %

Refresh
Save
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Effects of dye dilution

% of recommended concentration

100

75

50

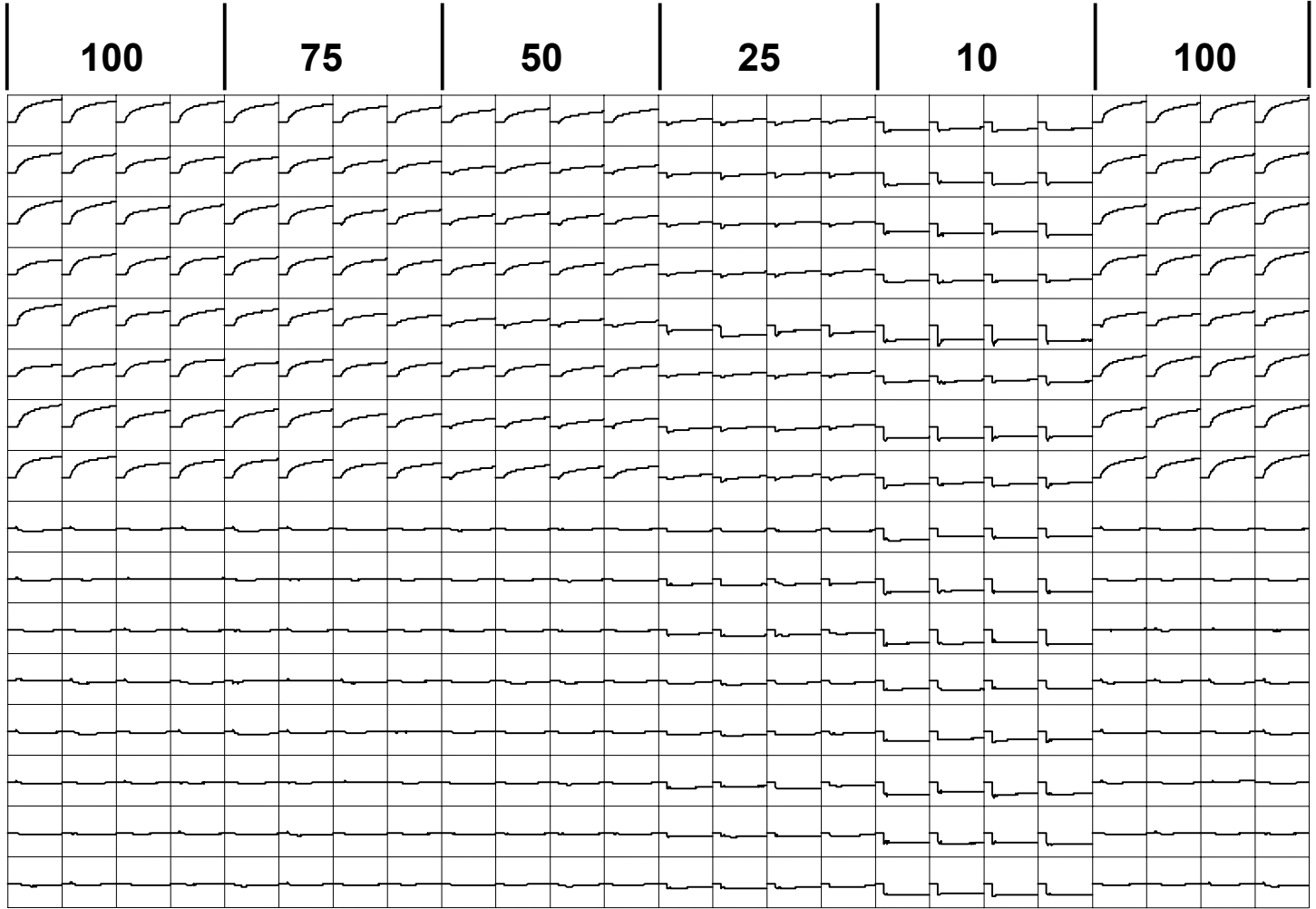
25

10

100

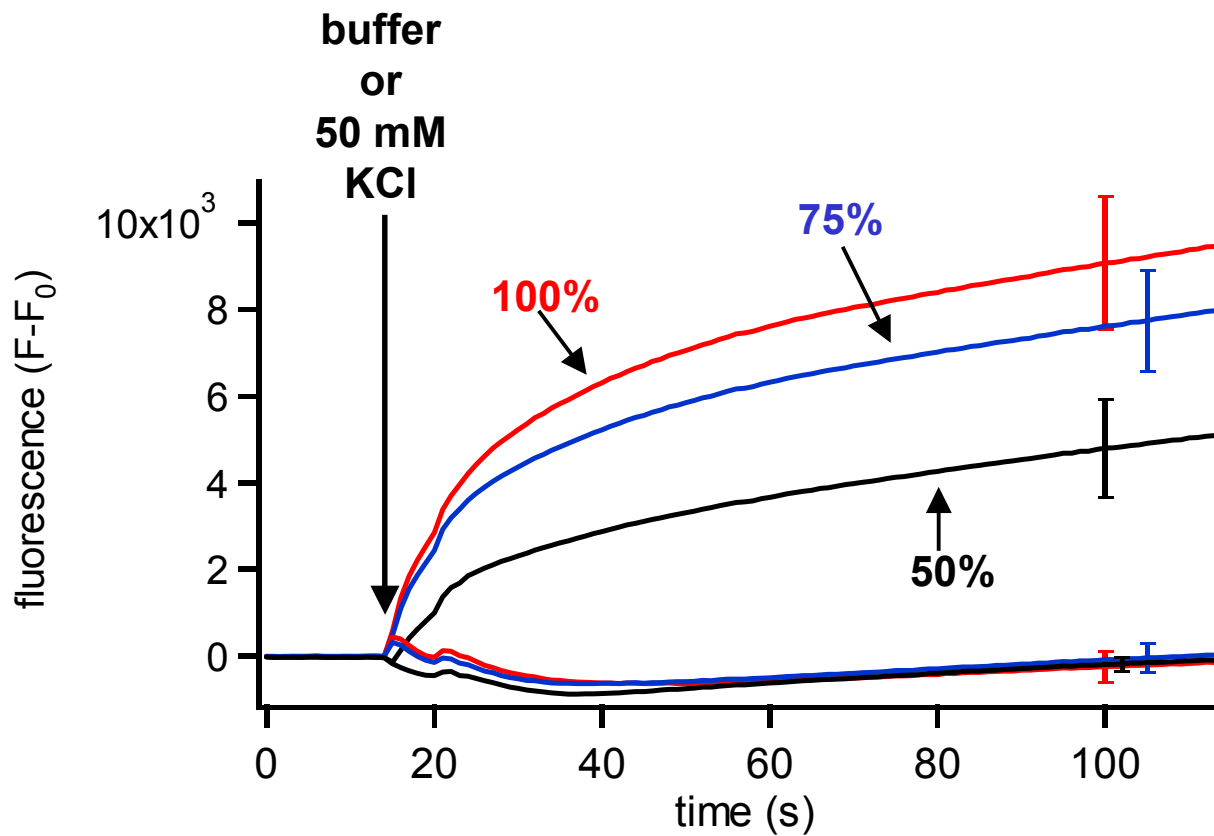
50 mM KCl
addition

Buffer
Addition



Range = (-12000, 14000)

signal vs dye dilution



Conclusions

MDC's membrane potential kit

The good

is easy to use- no wash
hyperpolarizations and depolarizations
rapid loading time
long recording window- 10->60 min
small addition artifacts

rapid response, tolerates DMSO

The bad

expensive
knowledge of mechanism

Other

valinomycin?

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