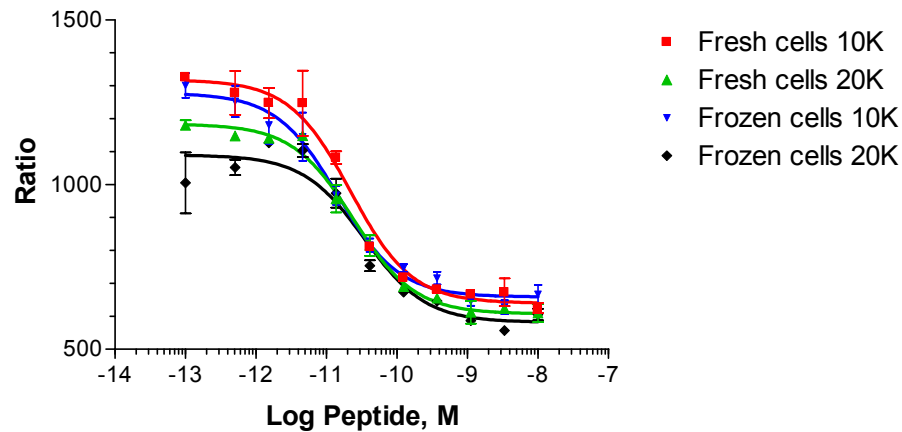


Cyclic AMP Assay: Frozen Cells

Cell Line #1 Agonist Response

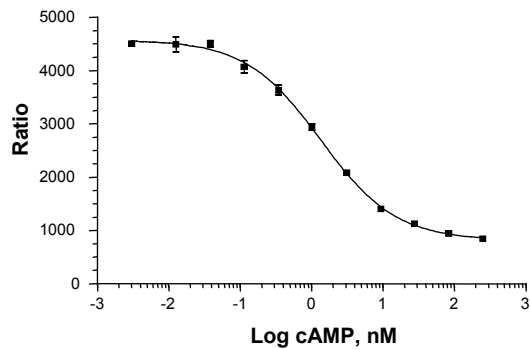


	Fresh cells 10K	Fresh cells 20K	Frozen cells 10K	Frozen cells 20K
BOTTOM	1318	1184	1278	1090
TOP	639.9	607.5	658.9	581.9
LOGEC50	-10.66	-10.62	-10.84	-10.46
EC50	2.2000e-011	2.4070e-011	1.4420e-011	3.4480e-011

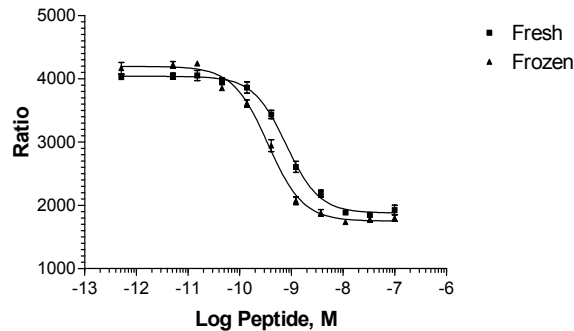
Excellent agreement of agonist EC₅₀

Cyclic AMP Assay: Frozen Cells

cAMP Standard Curve

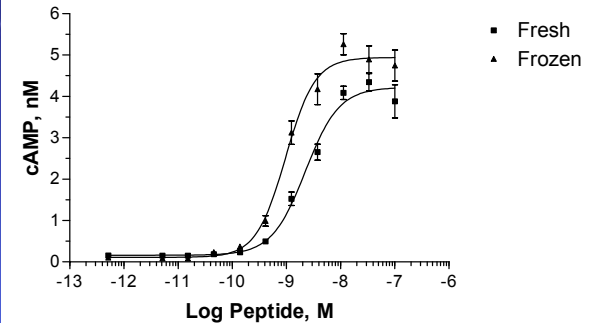


Cell Line #2 Agonist Response Curves



	Fresh	Frozen
BOTTOM	1877	1749
TOP	4038	4196
LOGEC50	-9.099	-9.453
HILLSLOPE	-1.356	-1.277
EC50	7.9530e-010	3.5210e-010

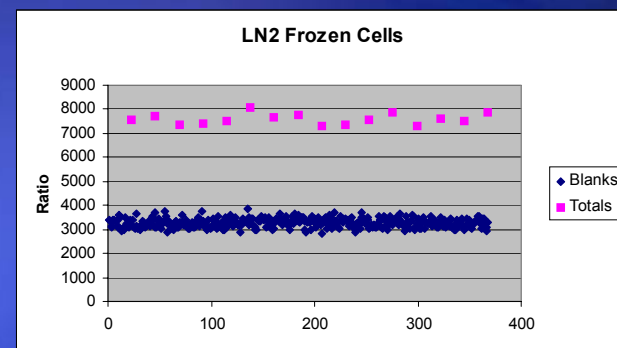
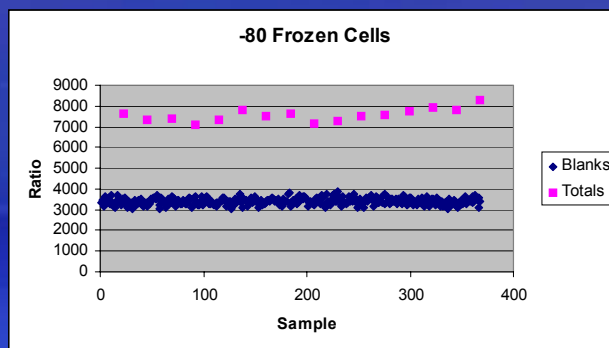
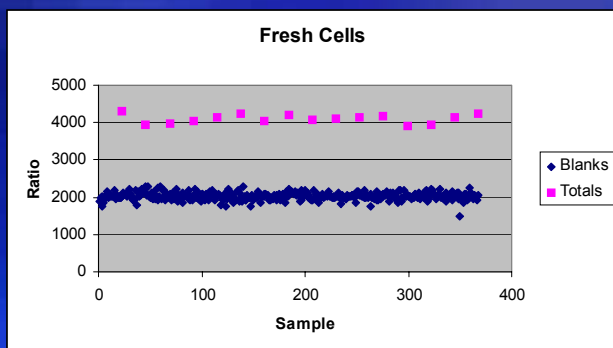
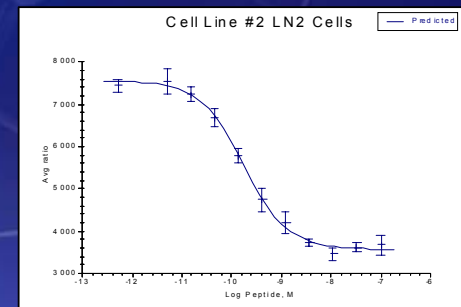
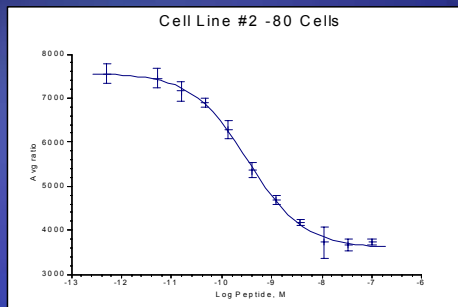
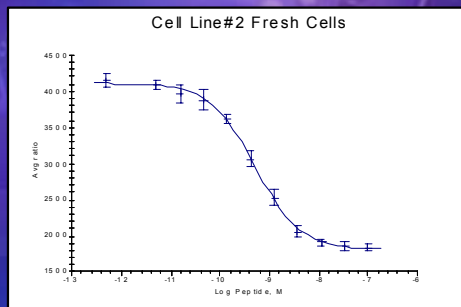
Cell Line #2 Agonist Response Curves



	Fresh	Frozen
BOTTOM	0.1644	0.1121
TOP	4.220	4.943
LOGEC50	-8.652	-9.017
HILLSLOPE	1.400	1.599
EC50	2.2270e-009	9.6090e-010

Excellent agreement of agonist EC₅₀

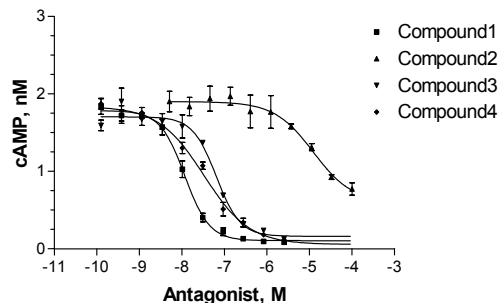
Frozen Cell cAMP Assay: LN₂ vs. -80° Storage



	Agonist EC50	Z'
Fresh Cells	5.07E-10	0.67
Frozen at -80°	3.35E-10	0.66
Frozen in LN ₂	1.76E-10	0.68

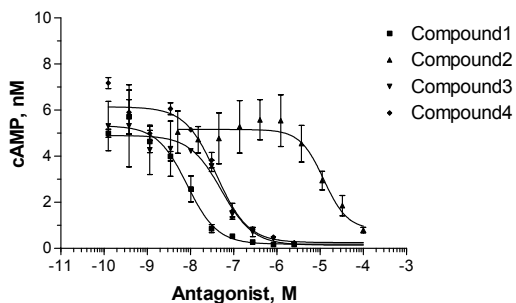
Frozen Cell cAMP Assay: Antagonists

Cell Line #2
Fresh Cells



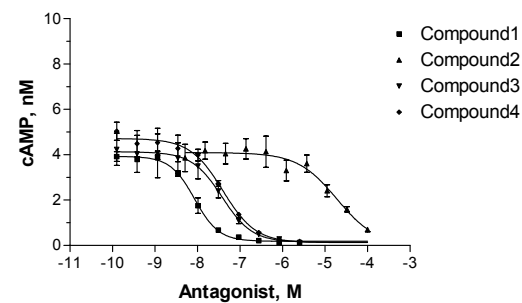
	Compound1	Compound2	Compound3	Compound4
BOTTOM	0.1074	0.6000	0.1639	0.05793
TOP	1.785	1.902	1.706	1.840
LOGEC50	-7.932	-4.910	-7.189	-7.479
HILLSLOPE	-1.500	-0.9477	-1.554	-0.8152
EC50	1.1700e-008	1.2300e-005	6.4650e-008	3.3170e-008

Cell Line #2
LN₂ Frozen Cells



	Compound1	Compound2	Compound3	Compound4
BOTTOM	0.1833	0.8175	0.1425	0.2549
TOP	5.324	5.160	4.902	6.143
LOGEC50	-8.074	-4.913	-7.281	-7.398
HILLSLOPE	-1.249	-1.582	-1.184	-1.198
EC50	8.4360e-009	1.2220e-005	5.2360e-008	4.0000e-008

Cell Line #2
-80° Frozen Cells



	Compound1	Compound2	Compound3	Compound4
BOTTOM	0.1845	0.005183	0.1435	0.1261
TOP	3.931	4.095	4.130	4.718
LOGEC50	-8.070	-4.720	-7.421	-7.404
HILLSLOPE	-1.521	-0.9375	-1.226	-1.129
EC50	8.5210e-009	1.9040e-005	3.7970e-008	3.9460e-008

	Compound1	Compound2	Compound3	Compound4
Fresh	1.17E-8	1.23E-5	6.47E-8	3.32E-8
Frozen in LN ₂	8.44E-9	1.22E-5	5.24E-8	4.00E-8
Frozen at -80°	8.52E-9	1.90E-5	3.80E-8	3.95E-8

Conclusions

- Frozen cells mimic fresh cells, even with cells that are slow to resurrect
- No difference between cells frozen for one month at -80° and -140°
- Membrane assay may be useful for direct comparisons of radioligand binding and functional responses
- The ability to suppress the observed basal cAMP accumulation in membrane preparations would be useful