

Evaluation of A New Long- wavelength Ca^{2+} Indicator (Calcium Red Dye)

Brief Introduction on Calcium Probes

Calcium is one of the most important 2nd messengers.

Intracellular calcium level can be elevated by activation of Gq-GPCRs or ligand-gated cation-channels

Fluo-3 and Fluo-4 provide a rapid and proven method of imaging intracellular calcium flux.

The changes in intracellular free Ca²⁺ concentrations can be measured by fluorescence microscopy, flow cytometry, fluorescence spectroscopy and fluorescence microplate readers.

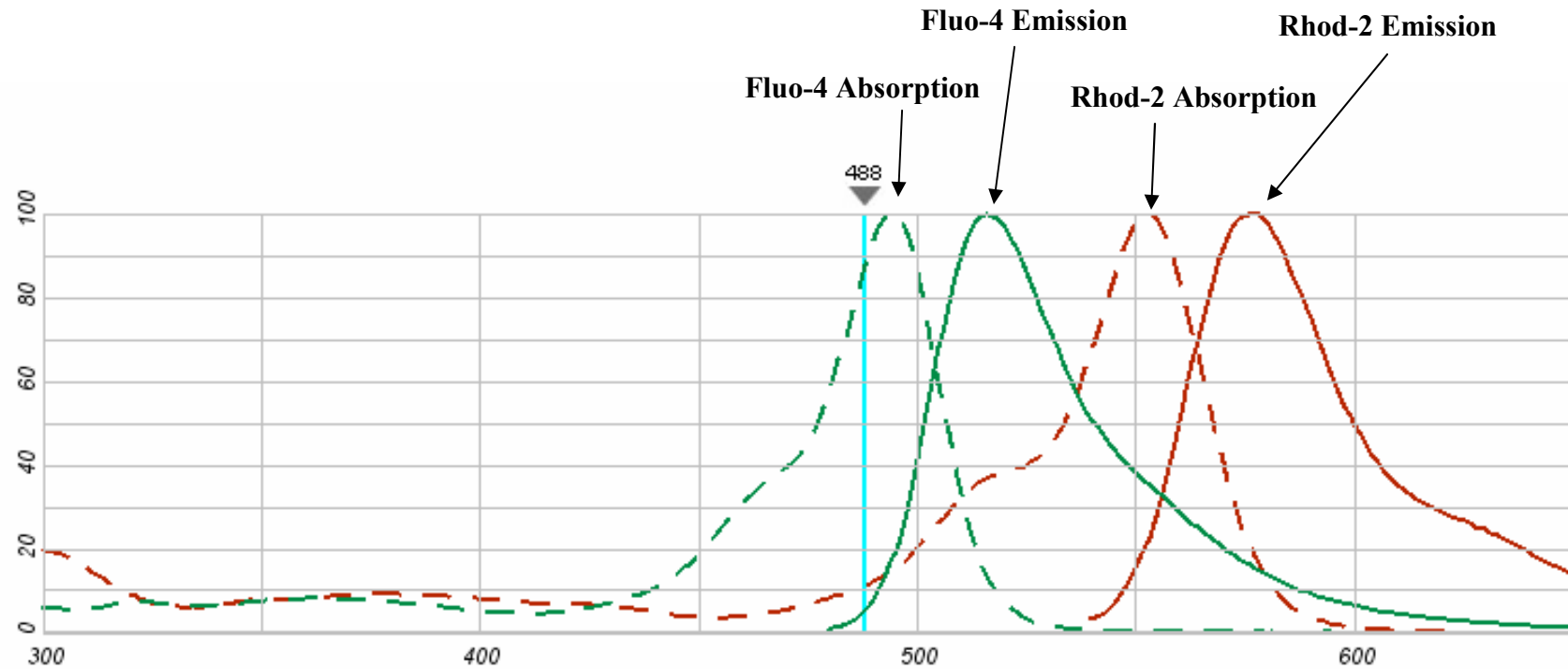
Potential Problems with Fluo-3 and Fluo-4:

a) autofluorescence from tissues or cell lines; b) fluorescence compounds interfere with the dye

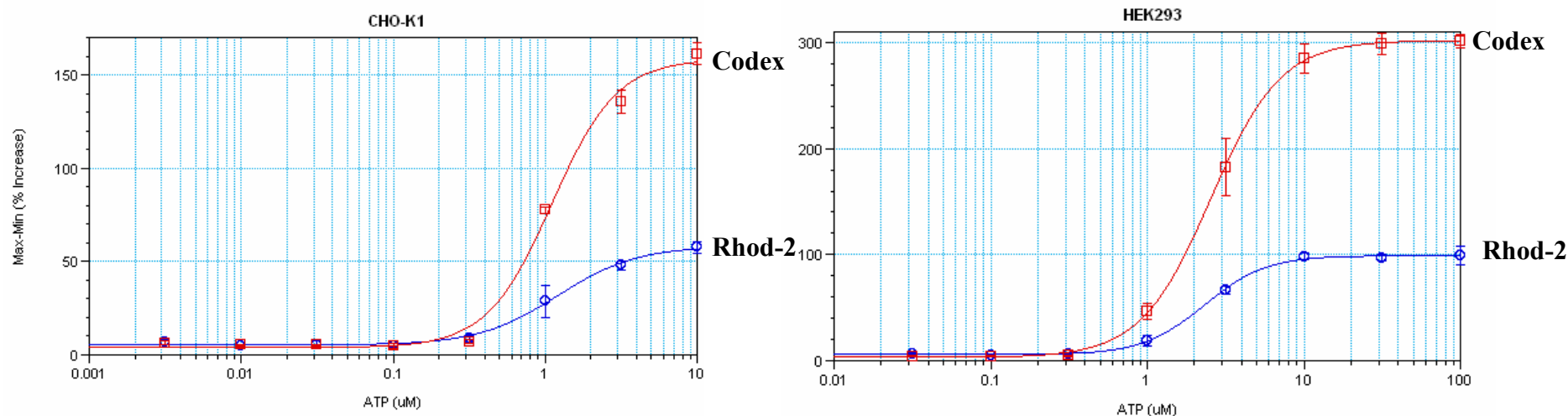
Fura-2 and Rhod-2 are alternative solutions

Codex Calcium Red Dye has similar Spectra as Rhod-2

Spectra of Fluo-4/Ca²⁺ and Rhod-2/Ca²⁺



Codex Red Dye Has Bigger Assay Window in Both CHO-K1 and HEK293 (Washing)



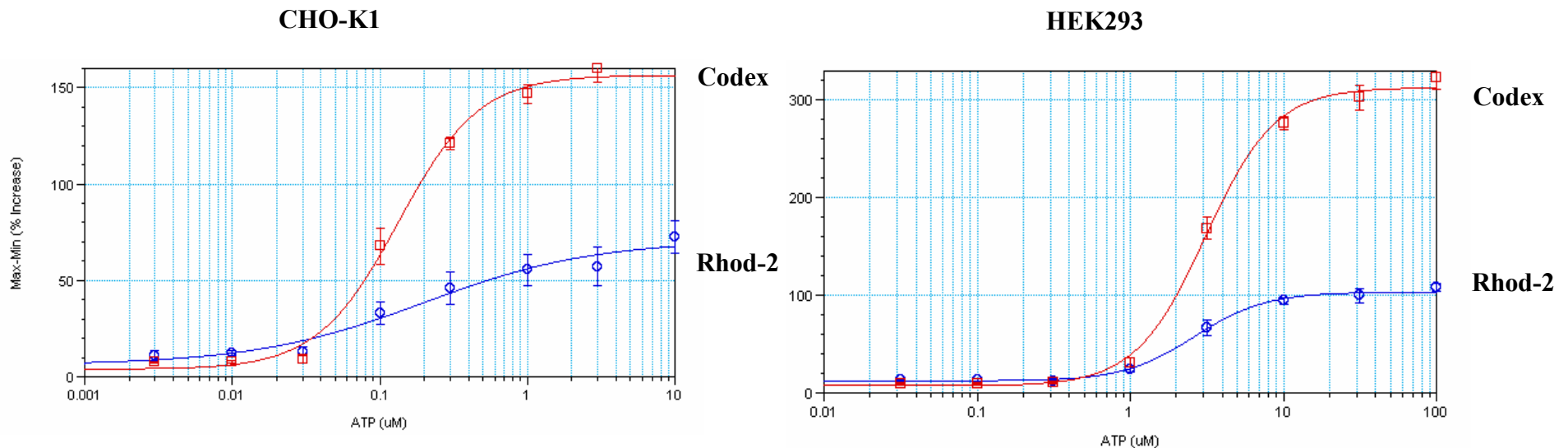
*ATP activates P2Y receptors in CHO and HEK293 cells

Dyes were loaded in the absence of growth medium. Free dyes were removed by washing.

Rhod-2: Ex: 550 nm, Em: 580 nm, cutoff 570 nm

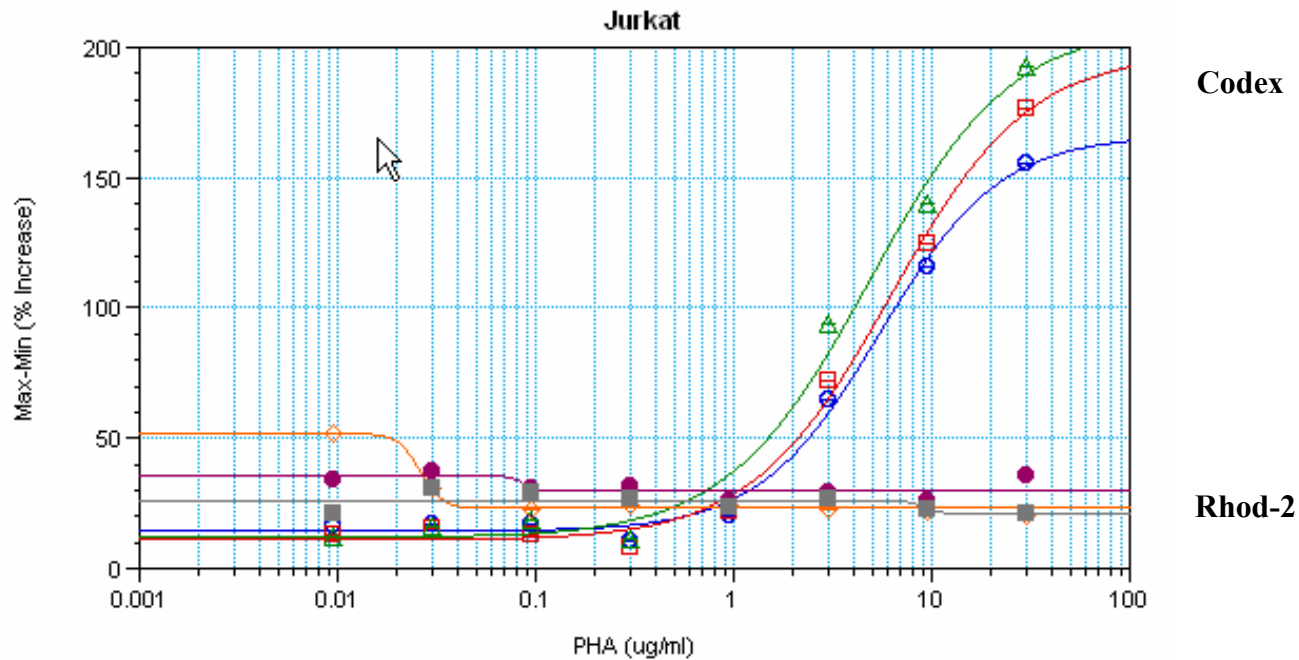
Codex Dye: Ex: 530 nm, Em: 570 nm, cutoff 550 nm

Codex Dye Has Bigger Assay Window in Both CHO-K1 and HEK293 (Non-Washing)



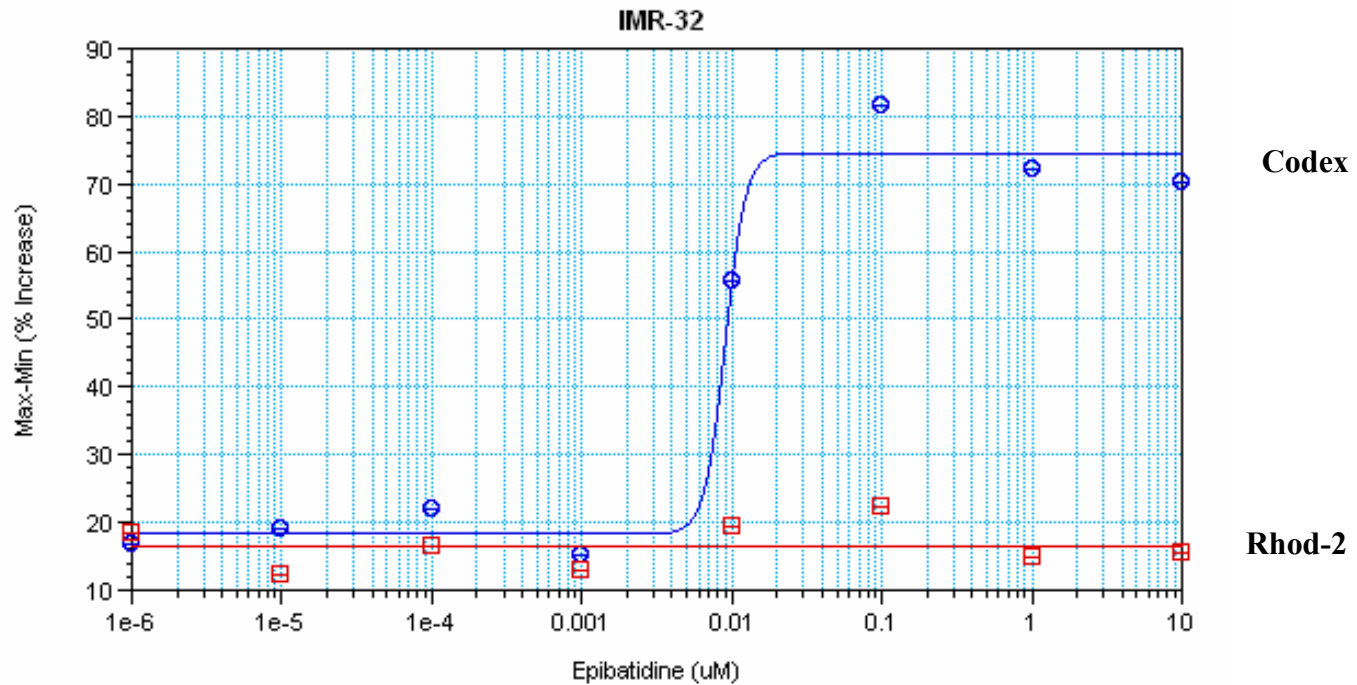
*ATP activates P2Y receptors in CHO and HEK293 cells
Dyes were loaded in the presence of growth medium. No washing steps involved
Rhod-2: Ex: 550 nm, Em: 580 nm, cutoff 570 nm
Codex Dye: Ex: 530 nm, Em: 570 nm, cutoff 550 nm

Jurkat Cells Stimulated with PHA (non-Washing)



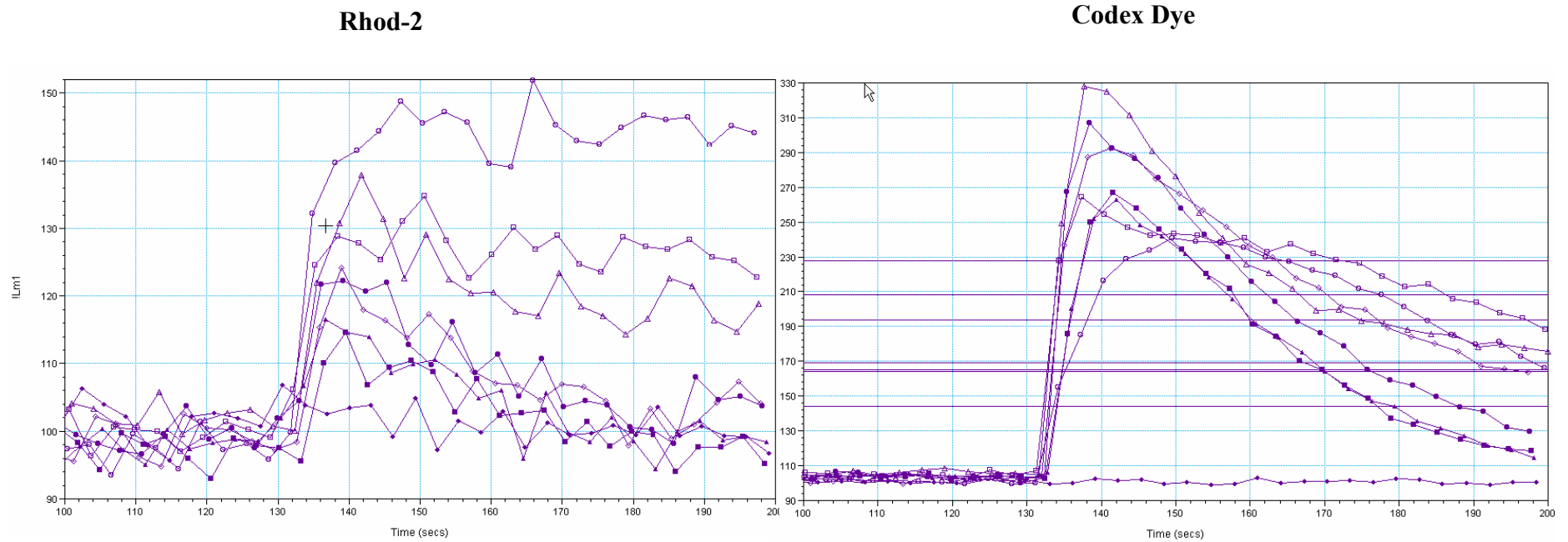
PHA → T-Cell Receptor (TCR) → Capacitative calcium entry (CCE)

IMR-32 Stimulated with Epibatidine (non-washing)



*Epibatidine activates nicotinic acetylcholine receptor (nAChR), a ligand gated ion-channel
IMR-32: human neuroblastoma cells

NG108-15 Cells Treated with ATP (non-washing)



NG108-15: Mouse neuroblastoma x Rat glioma hybrid. ATP activates both P2Y (Gq-GPCR) and P2X (ligand gated ion channel)

Summary

- **Codex Dye has bigger assay window than Rhod-2 in all the cells we tested so far.**
- **The assay window with Codex Dye in HEK293 cells is comparable with Fluo-4.**
- **Codex Dye can detect Ca²⁺ influx caused by both Gq-GPCR and ligand gated ion channel**