In vitro Pharmacology: Ca^{2+}- Flux Inhibition Fluorescence Assay in Jurkat Cells Example for ion channels inhibitors screening

- An increase in intracellular free Ca^{2+} concentration is a ubiquitous signalling mechanism that regulates a broad spectrum of cellular processes and is generally store-dependent.
- Store-operated Ca^{2+} release-activated Ca^{2+} channels (CRAC) are very high Ca^{2+} selective channels composed of two protein subunits: STIM1 Ca^{2+}sensor protein placed in the ER and ORAI1 Ca^{2+} channel protein in plasma membrane.
- In T cells, activation of CRAC leads to short term effects (reduced lymphocyte motility) and long term effects (altered gene expression & production of cytokines, ex. IL-2 and IFN-γ).
- Therefore, inhibition of CRAC (I_{crac}) is expected to modulate T cell activation and channels are potential target for new anti-inflammatory drugs as RA and psoriasis.
- Ca^{2+} -flux inhibition fluorescence assay in Jurkat cells is useful 384-plate format HTS screening assay for quick identification of STIM1/ORAI1 inhibitory compounds what is representative for CRAC inhibition in T cells.
- The assay has excellent correlation with patch clamp electrophysiology assay.

ASSAY PRINCIPLE

Intracellular Ca^{2+} is measured with Ca^{2+} sensitive dye.
Binding with Ca^{2+} ions results in fluorescent signal

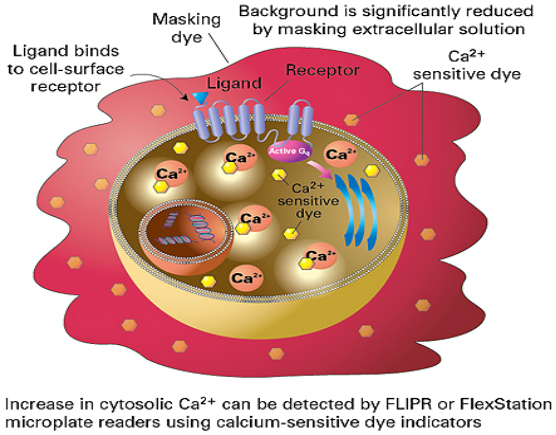

Figure 1. Principle of the Ca^{2+}-flux inhibition assay (figure source: Molecular devices).

QC parameters

Parameter	Ca-flux inhibition
Z'	>=0.5
Window (S/B)	Reproducible value
R^2	>=0.8
Hill slope	0.5-2
IC_{50}	Deviation=<3x
Range (Top-bottom)	Reproducible value (~30%-70%)

Assay results and data analysis

- Results are presented in a form of graphs and tables, with calculated IC_{50} values in nM.
- Method is programmed for PE Janus robotic system in WinPrep for Janus pipetting software.
- Control compound, YM-58483/BPT2 (Astellas), a potent inhibitor of CRAC channels, blocking thapsigargin-induced sustained calcium influx, is tested on every plate.
- Calculation of IC_{50} data, curves and QC analysis is made by using Excel tools and GraphPadPrism software.
- QC criteria parameters (Z', S:B, R^2, HillSlope) were checked for every IC_{50} curve.

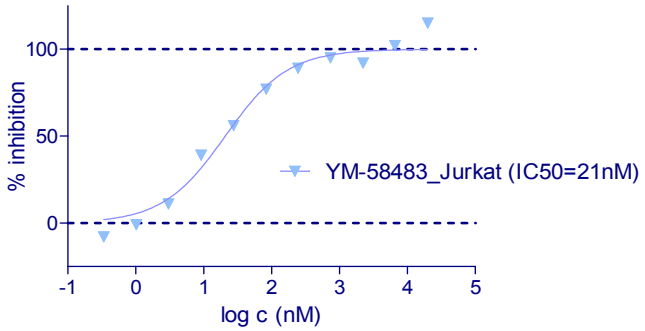

Figure 2. Ca^{2+}-flux inhibition profile of YM-58483, a potent inhibitor of CRAC channels.

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