

SOP-P057

Amgen Screening Project – Dye Preparations

Objective: To prepare JC1 and Redox staining solutions for staining HL60's for the Hypercyt/Cyan Screening.

Products: Calcein	Invitrogen	Cat# C3099 – 1ml
Mitosox	Invitrogen	Cat# M36008 – 10x50ug
mBBr	Invitrogen	Cat# M1378 – 25mg
JC1	Invitrogen	Cat# T3168 – 5mg
Valinomycin	Sigma	Cat# V0627 – 10mg

Procedure: JC-1 Dye, quantity for preparing ONE 384 well plate.

1. Stock concentration for JC1 is 5mM in DMSO
2. Ratio : 1ul of stock / 1ml of PBS with 0.1%BSA
3. Thaw one (9ul) aliquoted vial of JC1 from freeze. Add this to 9mls of PBSw/0.1% BSA. Mix well.

Procedure: Redox Dye, quantity for preparing ONE 384 well plate.

1. Stock concentration solution for calceinAM: 1 mM. in DMSO
mBBr: 40 mM in DMSO
Mito-sox: 50ug
2. **Mito-sox:** To each of two vials of mitosox, add 6.58ul of DMSO, Mix well.
3. **mBBr:** Take one (9ul) aliquoted vial out of freezer and thaw.
4. **CalceinAM:** ADD 1ul of stock to 30mls of the PBS /0.1% BSA , Mix well
5. Transfer 9mls of this solution into a 15cc conical tube
6. To this tube, add 9ul of Mito-sox and 9ul of mBBr.
7. This solution is referred to as the Redox Staining Solution.

Desired Final Concentrations:

JC-1	2.5uM	mBBr	40uM
Mitosox	10uM	CalceinAM	0.033uM
Valinomycin	1uM		

Created by: Gretchen Lawler

Verified by: _____ **Date:** _____

Print Name Sign Name