**Buffer Concentrations:**

Reagents

* 2M KCl
* 0.25M HEPES (pH 7.5)
* 2.5M Mannitol
* 0.2M Sodium succinate
* 1M MOPS (pH 7.5)
* 0.5M MgCl2
* 2.5M Sucrose
* 0.1M K2HPO4
* 0.1M DTT
* EGTA (380.35 g/mol)
* 0.00086 g ADP (427.2 g/mol)
* 1.502 g ATP (551.18 g/mol)

**Extraction Buffer A, 5x~ 50 ml**

50 mM HEPES, pH 7.5, containing 1 M mannitol,

350 mM sucr**o**se, and 5 mM EGTA

**Storage Buffer, 5x~ 25 ml**

50 mM HEPES, pH 7.5, c**o**ntaining 1.25 M sucr**o**se,

5 mM ATP, 0.4 mM ADP, 25 mM sodium succinate,

10 mM K2 HPO4 , and 5 mM DTT

**Albumin Solution 10 ml**

50 mg/ml delipidated bovine serum albumin

in water

**JC-1 Assay Buffer, 5x~ 25 ml**

100 mM MOPS, pH 7.5, containing 550 mM KCl,

50 mM ATP, 50 mM MgCl2 , 50 mM sodium succinate,

and 5 mM EGTA

**Recipes:**

**JC-1 Solution** (20 – 5ul aliquots, 5 mg/ml):

* Add 0.5 mg (0.0005 g) JC-1 to 90 ul DMSO
* Mix gently until dissolved
* Set p100/p200 to 100ul, suck mixture into pipet, determine volume (V)
* Add (1000 – V) ul DMSO to mixture
* Aliquot 5ul into separate 0.5ml pre-labeled tubes
* Store at -20ºC

**CCCP Solution** (10 – 100ul aliquots, 5mM):

* Add 1.02 mg (0.00102 g) CCCP to 900 ul DMSO
* Mix gently until dissolved
* Set p1000 to 1000ul, suck mixture into pipet, determine volume (V)
* Add (1000 – V) ul DMSO to mixture
* Aliquot 100 ul into 10 pre-labeled 0.5ml tubes.
* Store at -20ºC

**Fatty Acid Free BSA Solution** (10 – 340 ul aliquots, 50 mg/ml):

* Add 170 mg (0.170 g) to 3400 ul RNase/DNase free H2O
* Mix gently until dissolved
* Aliquot 340 ul into pre-labeled 1.5ml tubes
* Store at -20ºC

**Mitochondria Extraction Buffer A** (25 – 2ml aliquots, 5X):

* Add 10 ml 0.25 M HEPES (pH 7.5) to 10 ml H2O
* Add 20 ml 2.5M D-mannitol to mixture
* Add 7 ml 2.5M sucrose to mixture
* Add 0.0951 g EGTA to mixture
* Fill with H2O up to 50 ml
* Aliquot 2ml into pre-labeled 10ml conical tubes
* Store at -20ºC

**Mitochondria Storage Buffer** (50 – 100ul aliquots, 5X):

* Add 1000 ul 0.25M HEPES (pH 7.5) to 2500 ul 2.5M sucrose
* Add 625 ul 0.2M sodium succinate to mixture
* Add 500 ul 0.1M K2HPO4 to mixture
* Add 250 ul 0.1M DTT to mixture **(toxic)**
* Add 0.01378 g ATP to mixture
* Add 0.00086 g ADP to mixture
* Add H2O up to 5 ml (should be ~125 ul H2O)
* Aliquot 100 ul into pre-labeled 1.5ml tubes
* Store at -20ºC

**JC-1 Assay Buffer** (15 – 3.6ml aliquots, 5X):

* Add 14.85 ml 2M KCl to 14.85 ml H2O
* Add 5.4 ml 1M MOPS (pH 7.5) to mixture
* Add 5.4 ml 0.5M MgCl2 to mixture
* Add 13.5 ml 0.2M sodium succinate to mixture
* Add 0.103 g EGTA
* Add 1.4882 g ATP to mixture
* Aliquot 3.6 ml into pre-labeled 50ml conical tubes
* Store at -20ºC