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ASSAY REPORT: FRESH VS FROZEN UMBILICAL CORD TISSUE AND CELL SAMPLE

Date: January 18-2017
Report Created by: JEM

Cell Viability and Identity Protocol

- Pre-warmed water bath to 37°
- Add sample ID to 15ml conical tubes. One tube for each frozen vial.
- Add 10ml wash buffer to each 15ml conical
- Put frozen vial into holes in floater and put into water bath
- As soon as vial is 50% thawed, take out of water bath and dump into 15ml conical tube.
- With pipette, transfer the remaining liquid from vial into conical tube.
- Spin conical tubes 250g X 3min.
- Aspirate all liquid from tubes.
- Add 1ml of wash buffer and gently re-suspend with pipette.
- Transfer 300ul to pre-labelled tubes
- Add individual antibodies or premade cocktail to appropriate tubes.
- Tube1=unstained
- Tube 2= Isotype control
- Tube 3= Sample
- Incubate 30min 4°
- Add 4ml wash buffer
- Spin 250g X 3min
- Re-suspend in 500ul wash buffer
- Add 10ul of Accudrop Counting beads to Tube 3
- Run on flow cytometer immediately.
- Add 5ul DAPI 5 min prior to acquisition

Cat#	Vendor	Antibody	Fluor	ul/test
11-0909-41	ebioscience	CD 90	FITC	2.5ul
11-4714-41	ebioscience	IgG	FITC	2.5ul
12-1057-41	ebioscience	CD105	PE	2.5ul
12-4714-41	ebioscience	IgG	PE	5ul
25-9459-41	ebioscience	CD45	PECY7	2.5ul
25-4714-41	ebioscience	IgG	PECY7	2.5ul
17-0739-41	ebioscience	CD73	APC	2.5ul
17-4714-41	ebioscience	IgG	APC	0.625
		viability	DAPI	5ul

Reagents

Wash Buffer- 2.5%BSA in PBS (Core)

ASSAY RESULTS

Fresh Sample ID

Excluding RBC contamination, cellularity consisted of 9.7X10^5 viable cells per mL. Viability based on exclusion of DAPI equals 67.6%. Cellular phenotype consisted of:
CD90= 59.2%
CD105= 55.4%
CD73= 86.7%
CD45= 3.19%
Total Viable Meschencymal Stem Cell Count: 1,200,000 per mL. (This number is an approximation based on the use of bead markers. There is to be expected normal cell loss based on the steps to process the sample before Flow Cytometry testing. The 1,200,000 per mL number is the cell count that remains after testing protocol preparations.)

Frozen Sample ID

Excluding RBC contamination, cellularity consisted of 7.8X10^5 viable cells per mL. Viability based on exclusion of DAPI equals 43.1%. Cellular phenotype consisted of:
CD90= 28.0%
CD105= 35.1%
CD73= 84.4%
CD45= 4.06%
Total Viable Meschencymal Stem Cell Count: 800,000 per mL. (This number is an approximation based on the use of bead markers. There is to be expected normal cell loss based on the steps to process the sample before Flow Cytometry testing. The 800,000 per mL number is the cell count that remains after testing protocol preparations.)

James Marvin
Director, Flow Cytometry SRL