

Materials List for:

# Isolation of CD146<sup>+</sup> Resident Lung Mesenchymal Stromal Cells from Rat Lungs

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## Materials

Name	Company	Catalog Number	Comments
Neutral Protease	Worthington Biochemical Corporation	LS02104	Prepare on day of isolation and store at 4 °C until use
Collagenase I	Worthington Biochemical Corporation	LS004196	Prepare on day of isolation and store at 4 °C until use
DNAse I	Sigma-Aldrich	D5025	Prepare on day of isolation and store at 4 °C until use
Pentobarbital sodium (Euthanyl)	Bimeda-MTC Animal Health Inc, Dublin, Ireland	-	Use 0.2 ml for rat pups between 20-30 g; larger animals may need more.
Dulbecco's PBS + Sodium-pyruvate + Glucose	Life Technologies	14287-072	Very crucial to use this type of D-PBS, as the calcium and magnesium that are present in this D-PBS facilitate facilitate the enzymatic digestion
Ficoll-Paque PREMIUM (1.073 g/cm <sup>3</sup> )	GE Healthcare	17-5446-52	Density gradient media. To obtain a good interphase, it is crucial that the layering of the single cell suspension occurs at a >45° angle at very low speed, and that the subsequent centrifugation is done at 19 °C.
αMEM	Sigma-Aldrich	M8042	Warm in 37 °C water bath before use
200 mM L-Glutamine	Life Technologies	25030-164	
100x Antibiotic-Antimycotic	Life Technologies	15240-062	Penicillin/Streptomycin/Fungizone
M-280 Dynabeads	Life Technologies	11205D	Magnetic beads
biotinylated polyclonal rabbit-anti-mouse IgG	Dako, Agilent Technologies	E0464	Biotinylated secondary antibody that matches with the anti-rat CD146 antibody described below
DynaMag5-magnet	Life Technologies	12303D	Magnet that is recommended for use with Dynabeads. NOTE: magnet should be chosen based on the manufacturer's instructions of the magnet beads of choice.
TrypLE express	Life Technologies	12605-028	Gentle non-trypsin alternative, use 1 ml of TrypLE express/25 cm <sup>2</sup> surface area. After detachment, 10 ml L-MSC culture medium is sufficient to inactivate 3 ml TrypLE

anti-rat CD146 antibody	Lifespan Biosciences Inc.	C35841	12.5 µl per 0.5 x 10 <sup>6</sup> cells
Pentaspan (pentastarch solution)	Bristol-Myers Squibb Canada	-	Can be obtained through local blood donation services or hematology departments. Alternatively, one could use the PSI 20% Pentastarch solution (Preservation Solutions, PST001) diluted 1:1 with 0.9% NaCl.
Mr. Frosty freezing container	ThermoFisher Scientific	5100-0001	Improves viability when freezing L-MSCs overnight at -80 °C