

<u>Equipment needed for isolation and purification</u>	<u>Company</u>	<u>Item #</u>	<u>Cost (\$)</u>	<u>Units</u>	<u>Notes</u>
10cm x 20-25mm petri dish, sterile	Various				For initial shaking of cells; ≥20mm height recommended
50mL conical tubes, sterile	Various				For spinning cells down
70-75% Ethanol, ACS/USP (undenatured) grade	Various				For initial/final wetting/cleaning of fur, and cleaning tubing
70-75-micron filter, stainless steel/brass, 3" diam	Dual Mfg Co	US3-2005	40.00	1	Brass frame w/SS mesh; Mesh size 200 = 75 microns
95mm diameter petri dish, sterile	Fisher	S67962	8.30	25	For filtration; use with Dual Mfg filter only
Absorbent bench underpads, deluxe, 17"x24"	VWR	56616-018	122.01	300	Sufficient for mouse work; need 2/mouse
Analytical scale	Various				For measuring out collagenase powder
Autoclave tape, 1/4"-1/2" width	Various				For securing animal; sticks better to wet surfaces
BD Vacutainer 23g x 3/4" cannula	Fisher	02-665-38	67.30	50	May use cannula of choice, but 22-24ga recommended
Bright-line hemacytometer	Sigma	Z359629-1EA	166.50	1	For quantification and assessment of viability
Cell culture bottles, 100mL	Corning	1395-100	87.66	10	Standard solid screw-top OK; autoclave before use
Drierite dessiccant w/indicator, 454g (1lb)	Sigma	Z38961-454G	40.30	1	For long-term storage of collagenase powder stock
Dumont #7b medical forceps- curved/serrated, 11cm	FST	11270-20	40.00	1	For excision of liver- curved, serrated tip helps with grasping fibers.
Eppendorf 50mL Combitip (reagent reservoir)	Fisher	21-381-116	86.24	45	Sample pack is cheapest option- only need single 50mL tip
Fisher fiber optic light source w/dual light guide	Fisher	12-562-36	536.96	1	Single arm version also OK; lower-cost alternative: Item 12-563-502
Fisher variable-flow peristaltic pump, medium flow	Fisher	13-876-2	253.69	1.00	Need 1mL/min (low) and ≥ 10mL/min (high)
Hardened fine iris scissors, straight, 8.5cm	FST	14090-09	85.50	1	Two pairs recommended; non-hardened version (14060-09) = \$63.00
Ice bucket	Various				For keeping cells cold in-between washes and while counting
Isoflurane or other anesthetic	Various				Use anesthetic of choice; be aware of potential systemic effects
IV administration set	Fisher	14-841-96	98.27	50	Need only one set, for bubble trap
Kimberly-Clark sterilization wrap, 12"x12"	Fisher	066-673-5	95.09	1000	For sterilization of surgical tools
Luer fittings for tubing; plastic or metal	Various				For tubing & reservoir connections
Mediflex Sepp antiseptic applicators	Fisher	14-910-38	85.52	200	Any PVP iodine will work; for cleaning fur
Microcentrifuge tubes, 1.5-2.0mL, sterilized	Various				For holding collagenase aliquots
Serrated octagon straight forceps, 9.5cm	FST	11040-08	142.50	1	For general work; less expensive alternative = 11008-13
Small-diameter tubing for pump, clear	Various				Try to minimize length used/diameter depends on flow needed
Surgical board	Various				Will be wrapped in bench underpad; can use any stiff board
Surgical face mask	Various				For isolations performed in open air
Swinging-arm centrifuge w/50mL buckets	Various				Must be able to spin at ≤ 50 xg; refrigerated (4C) preferred
Waterbath	Various				Set to 37C

<u>Reagents needed for isolation and purification</u>	<u>Company</u>	<u>Item #</u>	<u>Cost (\$)</u>	<u>Units</u>	<u>Notes</u>
1x PBS, sterile, cell culture grade	Various				Used for rising plates after coating w/collagen
Acetic acid, 0.02N, sterile	Various				Used for diluting Collagen I solutions for coating plates
Autoclaved DD H2O	Various				Used for final pump tubing rinse
BD type I collagen, from rat tail, in soln, 100mg	Fisher	CB-40236	170.41	1	100mg bottles generally are ~3.5mg/mL; dilute before use
Collagenase Type IV, Worthington, 1g	Worthington	CLS-4	160.00	1	May wish to try sampling program and buy in 5-10g lots
Dexamethasone, cell culture tested, 25mg	Sigma	D4902-25MG	25.50	1	Any cell culture grade dex will work; make 100uM & 10uM stocks in EtOH
DMEM-low glucose, w/CaCl2 (200mg/L), 500mL	Cellgro	10-014-CV	80.59	6	CaCl2 ABSOLUTELY necessary for digestion
Fetal bovine serum, standard cell culture grade	Various				Used at 10% for initial plating and first 3-4 hours of culture
Hank's buffered salt solution w/0.5mM EGTA, sterile	Self-made				Based on Invitrogen #14175095, w/0.5mM EGTA & 25mM HEPES
HEPES, cell culture tested, 100g	Sigma	H4034-100G	83.20	1	Make 2M solution in DD H2O, pH 7.4@RT; sterile filter
Penicillin/streptomycin, stabilized, 100x, 100mL	Sigma	P4333-100ML	19.20	1	100x = 10K units penicillin/10mg streptomycin per mL
Trypan Blue, 0.4%, sterile, cell culture tested, 20mL	Sigma	T8154-20ML	13.10	1	For counting/viability assessment

<u>Tested, known working media for overnight culture</u>	<u>Company</u>	<u>Item #</u>	<u>Cost (\$)</u>	<u>Units</u>	<u>Notes</u>
DMEM, glucose- & phenol red- free, powder, for 10L	Cellgro	90-113-PB	45.35	1	Add NaHCO3, and L-gln at minimum before use; flexible media
DMEM-high glucose, w/L-Gln & Na-pyruvate, 500mL	Cellgro	10-013-CV	79.82	6	25mM glucose; presence of Na-pyruvate augments glycogen
DMEM-high/F-12 50/50 mix, 500mL	Cellgro	10-090-CV	101.53	6	Medium glucose alternative to DMEM-high
DMEM-low glucose, w/L-Gln & Na-pyruvate, 500mL	Cellgro	10-014-CV	80.59	6	Good general base medium, alone or in 50/50 mix with F-12
Ham's F-12, w/L-Gln, 500mL	Cellgro	10-080-CV	87.07	6	Cells tend to have lower glycogen when F-12 is used
William's Medium E, w/NaHCO3, w/o L-Gln, 500mL	Sigma	E4128-500ML	25.50	1	Classical, rich, moderate glucose hepatocyte medium; add L-gln

<u>Other useful equipment/reagents</u>	<u>Company</u>	<u>Item #</u>	<u>Cost (\$)</u>	<u>Units</u>	<u>Notes</u>
20mm OD amber screw thread vials, 5mL, w/caps	Sigma	Z9370-U	82.00	12	For storage of collagenase dry stock; wide mouth facilitates dispensing
AquaClear SF, waterbath preservative, 10g	LabECX	WP-000-153	20.00	1	Minimal-toxicity H2O preservative; use at 1g/L, works ≥ 2 months
BD Falcon cell strainer, 70um, sterile	Fisher	08-771-2	127.46	50	Alternative to Dual Mfg filter; slower and more expensive
Craftsman Professional #27801 1500W (adj) heat gun	Sears	00927801000	109.99	1	For drying Dual Mfg filter (lower heat)/backup sterilization
Fisher LED light source, edge clamp	Fisher	12-563-502	360.03	1	Lower-cost alternative to fiber optic light
IgG-Free, Protease-Free BSA, 10g	Jackson Immuno	001-000-161	64.00	1	Confirmed to not interfere with insulin action at up to 0.75%
Jiffy-jack apparatus positioner	Cole-Parmer	EW-08507-20	155.00	1	For positioning peristaltic pump
Retractor, 2 pronged, blunt, extra delicate, 13mm	FST	17021-13	79.50	1	For stretching out especially difficult portal veins to aid cannulation
Wheaton wide-mouth amber bottles with caps, 4oz	Sigma	Z263133-1PAK	67.50	24	For storage of collagenase aliquot bottles; fill with desiccant