

Isolation of Viable Adipocytes and Stromal Vascular Fraction from Human Visceral Adipose Tissue Suitable for RNA Analysis and Macrophage Phenotyping

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Materials

Name	Company	Catalog Number	Comments
0.2 mL PCR tubes	Axygen	PCR-02-C	RNase, DNase free and nonpyrogenic
1.5 mL microcentrifuge tubes	Axygen	MCT-150-C	RNase, DNase free and nonpyrogenic
10 mL serological pipettes	Corning	CLS4101-50EA	Individually plastic wrapped
10 µL universal pipet tip	Axygen	T-300-L-R	RNase, DNase free and nonpyrogenic
10 µL universal pipet tip	Axygen	T-300-R-S	RNase, DNase free and nonpyrogenic
1000 µL universal pipet tip	Axygen	T-1000-B-R	RNase, DNase free and nonpyrogenic
2.0 mL microcentrifuge tube	Axygen	MCT-200-C	RNase, DNase free and nonpyrogenic
200 µL universal pipet tip	Axygen	T-200-Y-R	RNase, DNase free and nonpyrogenic
2100 Bioanalyzer Instrument	Agilent	G2939BA	-
2101 Bioanalyzer PC	Agilent	G2953CA	2100 Expert Software pre-installed in PC
5 ml Round Bottom Polystyrene Test Tube	Corning	352003	Snap cap, sterile
50 mL centrifuge tubes	Corning	CLS430828-100EA	Polipropilene, conical bottom and sterile
Acid-guanidinium-phenol based reagent	Zymo Research	R2050-1-200	TRI Reagent or similar

Agilent RNA 6000 Nano Kit	Agilent	5067-1511	-
Agilent Small RNA Kit	Agilent	5067-1548	-
APC/Cy7 anti-human CD14 Antibody	BioLegend	325620	0.4 mg/10 ⁶ cells, present on monocytes/macrophages, clone HCD14
Baker	-	-	250 ml, non sterile
Bovine serum albumin	Sigma-Aldrich	A3912-100G	Heat shock fraction, pH 5.2, ≥96%
Chip priming station	Agilent	5065-9951	-
Collagenase type II	Gibco	17101-015	Powder
D-(+)-Glucose	Sigma-Aldrich	G8270-100G	Powder
Direct-zol RNA Miniprep	Zymo Research	R2051	Supplied with 50 mL TRI reagen
Dissecting forceps	-	-	Steel, serrated jaws and round ends
Dissection tray	-	-	Stainless steel
Ethyl alcohol	Sigma-Aldrich	E7023-500ML	200 proof, for molecular biology
FACS Flow Sheath Fluid	BD Biosciences	342003	-
FACS Lysing Solution	BD Biosciences	349202	-
FACSAria III Flow Cytometer/Cell Sorter	BD Biosciences	648282	-
FASCDiva Software	BD Biosciences	642868	Software v6.0 pre-installed
Hemocytometer	Sigma	Z359629-1EA	-
Manual cell counter	-	-	-
Mayo dissecting scissors	-	-	Stainless steel
Microcentrifuge	-	-	Adjustable temperature
Nanodrop spectrophotometer	Thermo Scientific	ND2000LAPTOP	-
Orbital shaker	-	-	Adjustable temperature and speed
P10 variable volume micropipette	Thermo Scientific-Finnpipette	4642040	1 to 10 µL
P1000 variable volume micropipette	Thermo Scientific-Finnpipette	4642090	100 to 1000 µL
P2 variable volume micropipette	Thermo Scientific-Finnpipette	4642010	0.2 to 2 µL
P200 variable volume micropipette	Thermo Scientific-Finnpipette	4642080	20 to 200 µL
PCR tube storage rack	Axygen	R96PCRFSP	-
PE/Cy5 anti-human HLA-DR Antibody	BioLegend	307608	0.0625 mg/10 ⁶ cells, present on macrophages, clone L243
PE/Cy7 anti-human CD45 Antibody	BioLegend	304016	0.1 mg/10 ⁶ cells, present on leukocytes, clone H130
Phosphate buffered saline	Sigma-Aldrich	P3813-10PAK	Powder, pH 7.4, for preparing 1 L solutions
Pipette controller	-	-	-
Red Blood Cells Lysis Buffer	Roche	11 814 389 001	For preferential lysis of red blood cells from human whole blood
Refrigerated centrifuge	-	-	Whit adapter for 50 mL conical tubes
Sterile Specimen container	-	-	-
Transfer pipette	Thermo Scientific-Samco	204-1S	Sterile
Trypan Blue	Gibco	15250-061	0.4% Solution
Tube racks	-	-	For different tube sizes
Vortex Mini Shaker	Cientifica SENNA	BV101	-