

Collagenase Digestion of PVAT for Adipocyte Isolation

- 1) Collect PVAT from anesthetized rats and place samples in cold KRBB (Krebs-Ringer Bicarbonate Buffer). Place samples on ice or in refrigerator. If working with more than one type of PVAT, always store the remaining samples in the refrigerator until ready to use.
- 2) Clean PVAT immediately in cold KRBB. Remove as much visible blood as possible and take the PVAT off the vessels.
- 3) Once cleaned, place tissue into a STERILE 1.5 mL tube.
- 4) Prepare 1 mg/mL collagenase solution with KRBB. You may need to add 4% BSA to the solution for adipocyte stability. Determine whether this will interfere with your reaction prior to adding it.
- 5) Add 1 mL collagenase solution to each tube containing PVATs to be digested. For superior mesenteric and aortic PVATs, chop into small pieces using scissors. Mesenteric PVAT does not need to be chopped prior to digestion.
- 6) Place tubes on rotisserie in incubator at 37°C for ~30 minutes.
- 7) Evaluate the digestion of the samples after 30 minutes. In most cases, aortic and superior mesenteric PVATs will need another 30 minutes for complete digestion. Mesenteric PVAT will typically need another 15 minutes of digestion time.
- 8) When digestion is complete, place tubes in microcentrifuge at 0.2 rcf for 5 minutes at room temperature.
- 9) Using a 23 gauge needle attached to a 1 mL syringe, remove the SVF from the bottom of each tube and discard.
- 10) Add 500 µL cold KRBB and finger flick to mix. Centrifuge at 0.3 RCF for 10 minutes at room temperature.
- 11) Discard supernatant.
- 12) Repeat steps 10 and 11 three more times for a total of 4 washes.
- 13) Carefully remove as much supernatant as possible after the final wash and add the appropriate buffer for your experiment.
- 14) Continue with your experimental protocol.

KRBB (1 L)

Reagent	Concentration	Amount
NaCl	135 mM	7.88 g
KCl	5 mM	0.373 g
MgSO ₄	1 mM	0.246 g
K ₂ HPO ₄	0.4 mM	0.070 g
glucose	5.5 mM	0.991 g
HEPES	20 mM	4.766 g
Antibiotic/Antimicrobial (Cellgro 30-004CL)		10 mL

pH to 7.4