

	SOP-BCR-4.12	<b>Preparation of Collagenase for Mammary Reduction Tissue (1 Liter)</b>	Author: S. Clouthier  Approved: M. Wicha 	Rev: 1.0	Issued: 09/24/98 Revised: 6/3/14
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**1.0 Purpose**

The purpose of SOP 4.12 is to describe how to prepare Collagenase.

**2.0 Scope**

SOP 4.12 is intended to cover all resources, personnel and equipment in the BCR laboratory.

**3.0 Materials**

No.	Name	Amt.	Description	Storage Location
1.0	Ham's F12	477 mL	Nutrient Medium	Cold Storage
2.0	DMEM	477 mL	Dulbecco's Modified Eagle Medium	Cold Storage
3.0	Collagenase	Use Chart Below	Enzymes	Fridge 1
4.0	HEPES	10 mL	Buffering Agent	Fridge 5
5.0	Insulin	5 mL	Hormone	Freezer 2
6.0	FBS	20 mL	Fetal Bovine Serum	Freezer 2
7.0	HC	0.5 mL	Hydrocortisone	Freezer 2
8.0	CT	0.1 mL (=100 µL)	Cholera Toxin	Freezer 2 in CT box
9.0	Antibiotic-Antimicotic	10 mL	Antibiotic	Freezer 2
10.0	Gentamicin	Use Chart Below	Antibiotic	Fridge 1 in antibiotics box on door

**4.0 Procedure**

4.1 **Be sure to check concentrations!** Depending on the concentrations the amount added may vary (to solve this, divide 299,000 by units/mg)

Collagenase:

155/mg = 1.929g/L

345/mg = .867 g/L

Gentamicin:

50mg/mL = 80uL

10mg/mL = 400uL

4.2 Add one 50 mL conical tube (already measured out) of collagenase into Ham's F12 bottle or DMEM bottle. Stir with automatic stirrer while adding other ingredients to other bottle.

4.3 **THE REST OF THE WORK MUST BE COMPLETED UNDER THE HOOD!**

4.4 Add the rest of the ingredients listed above into the other bottle.

4.5 Filter bottle containing collagenase **ONLY** into sterile, autoclaved bottle (**DO NOT FILTER BOTTLE WITH OTHER INGREDIENTS**).

4.6 Combine filtered contents with other bottle in the large, sterile container.

4.7 Redistribute mixture into the two smaller STERILE plastic bottles.

4.8 Label "Tissue Collagenase," add date, and add initials.

4.9 Store in Freezer 2.

**5.0 Applicable References**

Not applicable

**6.0 Change Description**

Revision	Date	Reference	Description of Change
1.0	6/3/14	TL	Updated locations and collagenase amount to add