

	SOP-BCR-4.17	<b>Preparing Cholera Toxin</b>	Author: S. Clouthier  Approved: M. Wicha 	Rev: 3.0	Issued: 09/24/98 Revised: 9/4/15
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### 1.0 Purpose

The purpose of SOP 4.17 is to describe how to prepare Cholera Toxin.

### 2.0 Scope

SOP 4.17 is intended to cover all resources, personnel and equipment in the BCR laboratory.

### 3.0 Materials

No.	Name	Description	Storage Location
1.0	CT	Cholera Toxin	Fridge #1, (026-314S) brown <i>Sigma</i> bottle in gold canister
2.0	HBSS	Hank's Balanced Salt Solution	Cold Storage (026-380C)
3.0	Syringe	BD 10mL syringe, cat# 309604	026-314S-A
4.0	Filter	0.22 µm MillexGV Filter Unit (SLGV033RS, cat# F-2975-3)	026-320S




### 4.0 Procedure

- 4.1 Source: Sigma Chemicals C 3012 (=C8052), 1 mg size.
- 4.2 Store at a stock concentration of **100 ug/mL (use at a final working concentration of 100 ng/mL)**
- 4.3 Add 1 mL of HBSS using a pipette, to the CT tube to dissolve CT
- 4.4 Transfer contents to 15 mL tube
- 4.5 Rinse CT tube with 1 mL HBSS to get all residues out of the tube and add to the 15mL tube.
- 4.6 Add 8 mL of HBSS to 15 mL tube (total of 10 mL of HBSS used)
- 4.7 Open filter packaging, but **DON'T** handle the filter
- 4.8 Attach the filter to syringe (**Make sure filter is on tight!**)
- 4.9 Remove the plunger from the syringe before adding contents from the 15mL tube
  - Add contents of the 15mL tube to syringe and filter into a second 15mL tube (**don't let contents leak in process, especially when inserting plunger!**)
- 4.10 Divide into 500uL aliquots and store in 1mL sterile freezing tubes.
- 4.11 These can be labeled and stored at -20 C in the CT box in freezer #2 (026-314S)

### 5.0 Applicable References

### 6.0 Change Description

Revision	Date	Reference	Description of Change
1.0	7/17/12	CL	Updated room location
2.0	11/4/13	TL	Updated locations and specific use concentrations
3.0	9/4/15	SK	Updated supplies needed (3.0 and 4.0)

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