

	SOP-BCR-4.6	Preparation of Insulin	Author: S. Clouthier Approved: M. Wicha	Rev: 4	Issued: 09/24/08 Revised: 10/2/15
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1.0 Purpose

The purpose of SOP 4.6 is to provide details on how to prepare insulin.

2.0 Scope

SOP 4.6 is intended to cover all resources, personnel and equipment in the BCR laboratory.

3.0 Materials

No.	Name	Description	Storage Location
1.0	Insulin	Hormone	Freezer #2
2.0	N HCl	Acid	Acid Cabinet
3.0	Sterile ddi water	Filtered double distilled water	Lab benchtop

4.0 Procedure

- 4.1 Source: Sigma Chemicals I-2643, 50 mg. size
- 4.2 Use at final concentration of 5 μ M/mL (stock concentration of 1 mg/mL)
- 4.3 Dilute 1M stock 1:10 for 0.1 N HCl (usually it is already made up in acid cabinet)
- 4.4 Pour wash right onto insulin in its vial and swirl to dissolve insulin – insulin **must** be fully dissolved before adding H₂O (you may need to add more N HCl – just enough to dissolve the insulin-.5 mL or .75 mL is enough!)
- 4.5 Measure out 49.5 mL or 49.25 mL sterile ddi water, depending on how much N HCl was added (i.e. if .5 mL added, then add 49.5 mL of water, and if .75 mL was added then add only 49.25 mL of water).
- 4.6 Add water/insulin mix to a 100 mL beaker and give a concentration of 1000 μ g/mL.
- 4.7 Dispense in 5 mL aliquots into sterile disposable 15 mL centrifuge tubes
- 4.8 Label with name of product, date, and your initials.
- 4.9 Store frozen at -20° C
- 4.10 Store remainder of aliquot (if any) at 4° C after thawing.
- 4.11 Use 500 μ L/100 mL medium to give a final concentration of **5 μ g/mL**.

5.0 Applicable References

6.0 Change Description

Revision	Date	Reference	Description of Change
3	5/18/12	TL	New insulin and amount
4	10/2/15	CC	Updated new amount to aliquot