



SOP-BCR-4.20

Preparation of ELISA Reagents/ Buffers

Author: S. Clouthier

Approved: M. Wicha

Rev:
1.0

Issued: 05/18/09
Revised: 7/17/12

1.0 Purpose

To prepare blocking buffer, secondary antibody diluent, and stop solution for ELISA's.

2.0 Scope

This procedure is used to prepare ELISA reagents prior to use in ELISA experiments.

3.0 Description

Preparation of solutions/ reagents from stock solution.

4.0 Materials and Reagents:

No.	Name	Description	Concentration	Storage Location
1.0	BSA	Albumin bovine	2% and 1% final	4°C refrigerator
2.0	PBS	Phosphate buffered saline	1X	026-328S Counter
3.0	Tween	Tween-20	0.05% final	Chemical Supply (026-314S)
4.0	Sulfuric Acid	18M Stock	1M final	Chemical Supply (026-314S)
5.0	Stir plate	Hot plate/ magnetic rod	N/A	Supply area
6.0	Filter system	0.44um Filter/ 60MM Prefilter	N/A	Supply area

5.0 Procedure:

5.1 Prepare Blocking Buffer (2% BSA/PBS):

- Obtain Albumin Bovine from 4°C refrigerator, weight out 10g, and place in 500ml beaker.
- Add 490ml 1X PBS to beaker.
- Place magnetic rod in beaker, put foil on lid, and put on hot plate until all contents are dissolved.
- Sterile filter contents in beaker through 0.45um sterile filter system. Be sure to add a 60MM prefilter to filter system before adding contents of beaker.

5.2 Prepare Second Antibody Diluent (1% BSA/PBS, 0.05% Tween):

- Weigh out 5g BSA and place in a 500ml beaker
- Add 495ml 1X PBS.
- Add 0.25ml Tween .
- Place magnetic rod in beaker, cover top with foil, and place on hot plate until all contents are dissolved.
- Sterile filter contents as above.

5.3 Prepare 1M Stop Solution:

- Add 27.8ml of 18M Sulfuric Acid to 500ml glass bottle.
- Using graduated cylinder, measure out 472.2ml distilled water and pour into the 500ml bottle containing sulfuric acid.
- Label bottle and store at room temp.

6.0 Reference(s)

Not Applicable.

7.0 Procedural Change Description

Rev Level	Date	Reference	Description of Change
1.0	7/17/12	CL	Added room locations