STANDARD OPERATING PROCEDURE

Title: Optimization of ELISA Reagents

Effective Date: _____

Approvals (Signature and Date):

_____	_____	_____
Responsible Department Head	Technical Authority	QA/QC Manager

1. PURPOSE

1.1 To determine the optimal concentration of a relevant capture antibody or soluble antigen.

1.2 To determine the optimal concentration of a lot of specific enzyme linked secondary antibody.

1.3 To determine the suitability for use of a lot of substrate buffer as measured against a lot of known performance.

1.4 To determine the suitability for use of a lot of PNPP substrate as measured against a lot of known performance.

2. SCOPE

2.1 To evaluate reagents to be used in quantitation of 12.8 antibody in solutions.

2.2 This assay can also be used to evaluate reagents for use in most ELISA procedures.

3. RESPONSIBILITY

3.1 All personnel performing optimization of ELISA reagents are responsible for adhering to this procedure.

4. REFERENCE AND APPLICABLE DOCUMENTS

4.1 TM-0060, ELISA for the quantitation of 12.8 antibody in solutions.

5. MATERIALS AND EQUIPMENT

5.1 See section 9.1

6. HEALTH AND SAFETY CONSIDERATIONS

6.1 See company safety manual.

7. DOCUMENTATION REQUIREMENTS

7.1 Laboratory Notebook

7.2 Attachment A. Reagent Qualification Form

8. GENERAL

8.1 New reagents should be qualified prior to use in an ELISA procedure.

8.2 By titering new or unknown reagents together with known reagents in a ELISA based assay a optimal concentration for use can be determined for the new component.

8.3 The suitability for use of new preparations can be determined by comparing new buffers or substrate preparations side by side with old preparations. The new preparations should give results similar or superior to the old lots.

8.4 ELISA plates should be covered during incubations to prevent evaporation and contamination.

9. PROCEDURE

9.1 Materials and Equipment

9.1.1 Microplate reader/recording spectrophotometer capable of reading @ 405 nm +/- 10 nm (Molecular Devices V-Max).

9.1.2 Multi channel micropipettor able to dispense up to 200 uL (8 Channel Finn pipette 50-300 ul).

9.1.3 Single channel micropipettor able to dispense up to 200 uL (Gilson 20-200 ul).

9.1.4 200 uL sized tips for micropipettors (Intermountain P-3200-1).

9.1.5 12 x 75 mm borosilicate tubes (VWR 60824-546).

9.1.6 15 mL conical tubes (Falcon 2095)

9.1.7 50 mL conical tubes (Falcon 2070)

9.1.8 1.0 mL serological pipets (Falcon 7520)

9.1.9 10 mL serological pipets (Falcon 7551)

9.1.10 Reagent troughs or square petri plates for use as reservoirs (Falcon 1003).

9.1.11 Polystyrene flat bottomed 96 well microtiter plate (Nunc maxisorb ELISA plates)

9.1.12 Vortex mixer (Vortex - Genie2)

9.1.13 ELISA plate Washer (Dynatech ultra wash 2)

9.1.14 Disposable sealing tape for 96 well plates (Corning #430454).

9.2 Reagents

9.2.1 Dulbecco's Phosphate buffered saline (D-PBS without Ca^{++} and Mg^{++}, JRH # 210-3025)

9.2.2 PT buffer = 0.5% tween 20 (Sigma # P-1379) in 1x D-PBS + 0.01% Thimerosal (Sigma T5125)

9.2.3 PB buffer = 1.0% Bovine serum albumin (Miles Fraction V reagent grade # 210-3025) in Phosphate buffered saline buffer + 0.01% Thimerosal (Sigma T5125).

9.2.4 AMP buffer 0.1 M 2-amino-2-mehtyl-1,3-propanediol buffer, in 0.01% $MgCl_2$ pH 10.3.

9.2.4.1 Dissolve 10.51 gm of 2-amino-2-methyl-1,3-propanediol (Sigma # A9754) + 100 mg $MgCl_2$ (Sigma M9272) per 1.0 L Deionized or distilled H_2O + 0.01% Thimerosal (Sigma T5125). Make 5.0 liters.

9.2.4.2 Assign a lot number to this batch of buffer and assure that the number is written on each bottle along with the date and the initials of the technician preparing the buffer.

9.2.4.3 Each lot of buffer will be compared against the previous lot as described in this procedure. If this is the reagent to be qualified then samples of both the new and old lot will be needed.

9.2.4.4 Store the AMP buffer at 5^{o}C and make a new lot once/month.

9.2.4.5 Other substrates or buffer systems (e.g., HRPO) can be evaluated using this protocol. Follow instructions in SOP for particular assay for reagent preparations.

9.2.5 PNPP substrate solution - Dissolve 1.0 MG/ML p-Nitrophenyl phosphate (Sigma 104-100 or Zymed # 00-2201) in amino-2-methyl-1,3-propanediol buffer from 5.2.4. Each new lot of powdered PNPP will be qualified according to this procedure by comparing it against a previous lot. If this is the reagent to be qualified then samples of both the new and old lot will be needed. (Store PNPP at -20^{o}C (+/-5^{o}C) in dark containers.)

9.2.6 Capture antibody or antigen used for coating plate. If this is the reagent to be qualified then samples of both the new and old lot will be needed.

9.2.7 Alkaline Phosphatase conjugated secondary antibody. If this is the reagent to be qualified then samples of both the new and old lot will be needed.

9.2.8 Bicarbonate buffer - 0.05 M pH 9.6 (Sigma # C-3041) make from powder; store at Rt for up to 6 months.

9.2.9 Primary antibody or antigen for which the particular assay being evaluated is designed. If this is the reagent to be qualified then samples of both the new and old lot will be needed.

9.3 Evaluation of optimal concentrations of capture antibody (or antigen).

9.3.1 Prepare 1.5 mL of a 20 ug/mL solution of capture antibody or soluble antigen in Bicarbonate buffer.

9.3.2 Add 100 ul of bicarbonate buffer to each well of a 96 well ELISA plate.

9.3.3 Add 100 ul of coating material from 5.3.1 to each well of the first row of the ELISA plate. Titrate this material down the rows of the plate discarding the 100 ul of excessl material remaining from the last row.

9.3.4 Allow plate to incubate at 2-8^{o}C for 18-22 hr.

9.3.5 Wash the plates 3 times with PT buffer by sequentially dispensing >200 ul of buffer into the plate and aspirating off (repeated a total of three times using the plate washer).

9.3.6 Block the plates by adding 100 ul of PB buffer to each well.

9.3.7 Allow plates to incubate for 1.0 hour at R.T. and wash the plates with PT buffer as follows:

9.3.7.1 Wash 3 times as described in 5.3.5. Fill the plate with PT buffer.

9.3.7.2 Allow the plate to sit at R.T. for 5 minutes.

9.3.7.3 Wash 3 more times as described in 5.3.5.

9.3.8 Add 100 ul of PT buffer to each well of the plate.