Elisa protocol flow sheet

Name:
Date:

I. Hazard Assessment

To protect yourself from any possible hazards associated with this task wear eye protection. You should also wear latex, nitrile, or vinyl gloves and a lab coat with long sleeves. To protect your legs and feet wear closed shoes and long trowsers. Do not wear sandles, shorts or a short skirt. Wash your hands before eating and when leaving the laboratory. You should review the MSDS for any chemical used in this procedure. In case of a spill with a toxic chemical remove all contaminated clothing and wash affected areas with copious quantities of water. Check location of the nearest safety shower. Eyes should be washed copiously for 15 minutes.

When preparing 0.6 N Hydrochloric acid solution wear goggles that form a seal and nitrile gloves. Always add the acid to the water, not vice versa.

This protocol is based on the Maine Biotechnology Services ELISA protococol for SeM.

II. Reagents and supplies

Reagent	Amount	Separate SOP?	Ready
Coating buffer (0.15 M PBS, pH 7.6)	1 liter	Yes	____
Capture Antibody - MAB212 diluted in coating buffer (2 ug/mL)	10 mL	Yes	____
Wash Buffer (0.15 M PBS, 0.05% tween 20)	1 liter	Yes	____
Blocking Buffer (1% Non Fat Dried Milk (NFDM) in 0.15 M PBS)	100 ml	Yes	____
Sample: phagelysin digested sample, variable dilution profiles, 50 uL per well,	as needed	Yes	____
Detector Antibody MAB211-biotin diluted in blocking buffer (0.5 ug/mL)	10 mL	Yes	____
Tracer Streptavidin Horse Radish Peroxidase (HRP) diluted in wash buffer (1:10,000)	10 mL	Yes	____
Substrate, which is turned blue by HRP, TMBW (undiluted - single step reagent)	10 mL	No	____
Stop solution which causes blue to yellow color change (0.6 N Hydrochloric acid)	10 ml	No	____
Material			
ELISA Plates			____
Plate washer			____
Multichannel pipette			____
Unichannel pipettes			____
Pipette tips			____
Reagent tubes			____

Reagent boats ____

Comments:

III. **Coating Plates**

Label Plates ____
Dispense 50 ul of 2 ug/ml MAB212 capture antibody to all wells** ____
Cover and refridgerate at 4 C overnight ____

***Use Multichannel pipette*

Comments:

IV. **Wash Plates post-coating**

Wash # 1with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Flick then blot dry ____

Comments:

V. **Blocking**

Prepare blocking buffer (1 g NFDM in 100 mL PBS) ____
Label expiration date 1 week from date of preparation ____
Dispense 300 ul of Blocking Buffer (1% Non Fat Dried Milk in 0.15 M PBS) to all wells** ____
Refridgerate any excess blocking buffer ____
Incubate plates at room temperature for 1 hour or overnight at 4 C ____

***Use Multichannel pipette*

Comments:

VI. **Wash Plates post blocking**

Wash # 1with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____

Flick then blot dry ____

Comments:

VII. **Arrangent of samples by serial dilution 1:2 (or 1:6)**

Prepare phagelysin digested samples/controls in dilution tubes, label and record on summary sheet ____

Dispense 50 ul of 0.15 M PBS to all wells ____

Add 50 ul (or 10 ul) of samples to rows in column 1* ____

Transfer 50 ul (or 10 ul) to column 2** ____

Repeat transfer through row 12** ____

Discard the last 50 ul (or 10 ul) from row 12 ____

Incubate plates with rotation at 37 C for 30 minutes or at room temp for 1 hour ____

During this incubation, prepare a 0.5 ug/ml solution of the detector antibody MAB211P-biotin, in blocking buffer (1% NFDM in 0.15 M PBS; see separate SOP) ____

**Use single channel pipette*
***Use Multichannel pipette, mixing 8 X before each transfer*

Comments:

VIII. **Wash Plates post sample incubation**

Wash # 1with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____

Flick then blot dry ____

Comments:

IX. **Addition of Detector Antibody MAB211P-Biotin**

Pipet 50 ul well of the detector antibody (MAB211P-biotin) to each well** ____

Incubate at 37C for 30 minutes or 1 hour at RT, with rotation if possible. ____

During this incubation, prepare a 1:10,000 dilution of the Streptavidin HRP in wash buffer. See separate SOP ____

***Use Multichannel pipette*

Comments:

X. **Wash Plates post incubation with MAB211P-Biotin Detector**

Wash # 1 with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Flick then blot dry ____

Comments:

XI. **Addition of Streptavidin HRP**

Pipet 50 ul of the Streptavidin HRP at 1:10,000 to each well** ____
Incubate at 37C for 30 minutes or 1 hour at RT, with rotation if possible. ____

***Use Multichannel pipette*

Comments:

XII. **Wash plates post incubation with Streptavidin HRP**

Wash # 1 with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Wash # 2 with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Wash # 3 with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Wash # 4 with automatic plate washer (0.15 M PBS, 0.05% tween 20), fill 300 uL ____
Flick then blot dry ____

Comments:

XIII. **Addition of TMBW color reagent**

Add 50 ul of the TMB to each well on plate ** ____
Start timer for 10 minutes and cover plate** ____
After 10 minutes, stop the enzyme reaction by adding 50 ul of 0.6N HCL ____

Read the plate at A(450 nm) ____

******_Use Multichannel pipette_

Comments: