

# ELISA PROTOCOL

## ANTIGEN SPECIFIC IgG<sub>1</sub>/IgG<sub>2a</sub>



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**Used by the Laboratory of William C. Gause, Ph.D.**

STEP	μL/WELL	DILUTION	BUFFER	INCUBATION
Coat with Diluted Antigen – Hp, Tm, or Nb Extract	50	5μg/mL	BBS	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Block the Plate	50		PCS	30 min. RT
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Apply Samples Samples - Start with 1:20 and do 1:4 serial dilutions	50		BBS + Tween	2 hrs. RT Overnight 4°C
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Incubate with Detection Antibody - α-mouse IgG-AP IgG <sub>1</sub> - Southern Biotechnology Associates Cat # 1070-04 IgG <sub>2a</sub> - Southern Biotechnology Associates Cat # 1080-04	50	1:1000	BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 3 times	300		ddH <sub>2</sub> O	
Add Substrate and Develop - MUP MUP - Sigma Cat # M-8883	50	5mg/100mL	MUP Buffer	Until Highest Standard Reaches 2800 F.U.
Detect Fluorescence on ELISA Plate Reader				

ELISA protocol developed using Immulon IV plates from Dynex, Cat #3855.

### Manufacturer Phone Numbers:

1. Southern Biotechnology Associates, Inc. – 205.945.1774
2. Sigma – 800.325.3010
3. Dynex – 703.631.7800

**NOTE:** Protocol does not work with IgG<sub>2a</sub>-Tm.



# ANTIGEN-SPECIFIC IgG<sub>1</sub>/IgG<sub>2a</sub> ELISA BUFFERS

## 1. Borate Buffered Saline (BBS)

Begin with 5L ddH<sub>2</sub>O in 6L Erlenmeyer flask.

Add the following slowly with stirring:

H<sub>3</sub>BO<sub>3</sub> : 61.8 g

NaCl : 43.8 g

Adjust pH to 8.5 with 1N NaOH.

Bring volume to 6L with ddH<sub>2</sub>O.

Store at Room Temperature (RT).

## 2. BBS + Tween

Add 10mL of 1% Tween-20 stock to 500mL BBS.

Store at Room Temperature.

## 3. Protein Carrier Solution (PCS)

Add 5mL Fetal Bovine Serum to 500 mL BBS

Add 5mL 10% Sodium Azide.

Store at 4°C.

## 4. MUP Buffer

Begin with 5L ddH<sub>2</sub>O in 6L Erlenmeyer flask.

Add 25.2g NaHCO<sub>3</sub> and 1.21g MgCl<sub>2</sub> • 6H<sub>2</sub>O

Adjust pH to 9.7 with 1N NaOH.

Bring volume to 6L with ddH<sub>2</sub>O.

Store at Room Temperature (RT).