

ELISA PROTOCOL

HP- SPECIFIC IgE



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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 ES	50	5μg/mL	BBS	2 hrs. RT Overnight 4°C
Wash Plates 5 times	300		ddH ₂ O	
Block the Plate	50		PCS	30 min. RT
Wash Plates 5 times	300		ddH ₂ O	
Apply Samples Samples - Start with 1:5 and do 1:4 serial dilutions	50		BBS + Tween	2 hrs. RT Overnight 4°C
Wash Plates 5 times	300		ddH ₂ O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 5 times	300		ddH ₂ O	
Incubate with Detection Antibody - Biotin α-mouse IgE IgE – Pharmingen Cat. # 553419	50	1:250	BBS + Tween	1 hr. RT
Wash Plates 5 times	300		ddH ₂ O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 5 times	300		ddH ₂ O	
Incubate with 2° Antibody – Streptavidin-AKP SA-AKP – Pharmingen Cat. # 554065	25	1:1000	BBS + Tween	1 hr. RT
Wash Plates 5 times	300		ddH ₂ O	
Incubate with Wash Buffer	50		BBS + Tween	30 min. RT
Wash Plates 5 times	300		ddH ₂ 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. Pharmingen – 877.232.8995
2. Sigma – 800.325.3010
3. Dynex – 703.631.7800



HP-SPECIFIC IgE ELISA BUFFERS

1. Borate Buffered Saline (BBS)

Begin with 5L ddH₂O in 6L Ehrlenmeyer flask.

Add the following slowly with stirring:

H₃BO₃ : 61.8 g

NaCl : 43.8 g

Adjust pH to 8.5 with 1N NaOH.

Bring volume to 6L with ddH₂O.

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 ddH₂O in 6L Ehrlenmeyer flask.

Add 25.2g NaHCO₃ and 1.21g MgCl₂ • 6H₂O

Adjust pH to 9.7 with 1N NaOH.

Bring volume to 6L with ddH₂O.

Store at Room Temperature (RT).