NOTEBOOK-READY PROCEDURE

Sandwich ELISA Procedure

Experiment/Project #: _____

Name(s): _____

Date(s): _____

Material	Company/Catalog #/Lot #
Plate:	
Coating Antibody:	
Antigen/Sample:	
Secondary Antibody:	
Tertiary Antibody:	
Wash Solution:	
Blocker:	
Substrate:	
Coating Solution:	
Antibody Diluent:	
Stop Solution:	
Equipment	**Company/Catalog #/Lot #**
Plate Washer:	
Plate Reader:	

1. **DETECTION SYSTEM:** ☐ Chemiluminescent ☐ Colorimetric ☐ Fluorescent

2. **PLATE TYPE (96 WELL)**

   ☐ Clear ☐ Black ☐ White

   ☐ Flat Bottom ☐ U Bottom ☐ C Bottom

   ☐ High Binding ☐ Medium Binding ☐ Other: _____

3. **ANTIBODY COATING**

   1 hour at room temperature (RT) or overnight 4°C Temperature: _____

   Coating Antibody: _____ Concentration: _____

   Coating Solution: _____ Volume/Well: _____

   Antibody: _____ ☐ Polyclonal ☐ Monoclonal

   Antibody Purity: ☐ Unpurified ☐ Serum ☐ Protein A/G ☐ Antigen Affinity ☐ Ascites

   Actual time: Start - _____ AM/PM End - _____ AM/PM Total - _____

4. **BLOCK PLATE**

   1 hour at RT or overnight 4°C Blocker: _____ Temperature: _____

   Volume/Well: _____

   Actual time: Start - _____ AM/PM End - _____ AM/PM Total - _____

5. **ANTIGEN/SAMPLE INCUBATION**

   30 – 60 minutes at RT or overnight 4°C Temperature: _____

   Antigen: _____ Diluent: _____

   Antigen Concentration: _____ Volume/Well: _____

   Actual time: Start - _____ AM/PM End - _____ AM/PM Total - _____

6. **WASH PLATE**

Wash Solution: _____ Volume/Well: _____

Actual Time: Wash 1 - _____ Wash 2 - _____ Wash 3 - _____

7. **PRIMARY ANTIBODY INCUBATION**

30 – 60 minutes at RT or overnight 4°C Temperature: _____

Antibody: _____ ☐ Polyclonal ☐ Monoclonal

Antibody Diluent: _____

Conjugate: ☐ N/A ☐ HRP ☐ AP ☐ Other Tag: _____

Antibody Purity: ☐ Unpurified ☐ Serum ☐ Protein A/G ☐ Antigen Affinity ☐ Ascites

Antibody Concentration: _____ Dilution: _____

Actual Time: Start - _____ AM/PM End - _____ AM/PM Total - _____

8. **WASH PLATE** ☐ N/A

Wash Solution: _____

Actual Time: Wash 1 - _____ Wash 2 - _____ Wash 3 - _____

9. **SECONDARY ANTIBODY INCUBATION**

30 – 60 minutes at RT or overnight 4°C Temperature: _____

Antibody: _____ ☐ Polyclonal ☐ Monoclonal

Antibody Diluent: _____

Conjugate: ☐ HRP ☐ AP ☐ Other Tag:_____

Antibody Purity: ☐ Unpurified ☐ Serum ☐ Protein A/G ☐ Antigen Affinity ☐ Ascites

Antibody Concentration: _____ Dilution: _____

Actual Time: Start - _____ AM/PM End - _____ AM/PM Total - _____

10. **WASH PLATE** ☐ N/A

Wash Solution: _____

Actual Time: Wash 1 - _____ Wash 2 - _____ Wash 3 - _____

11: **SUBSTRATE INCUBATION**

Substrate Name: _____ ☐ Colorimetric ☐ Chemiluminescent ☐ Fluorescent

Recommended development time: _____ minutes Actual Time: _____ minutes

Stop Solution: _____ ☐ N/A

12. **DATA ANALYSIS**

Time: _____ minutes Slit Width:_____ Time/Well: _____

Computer File Name: _____ Location: _____
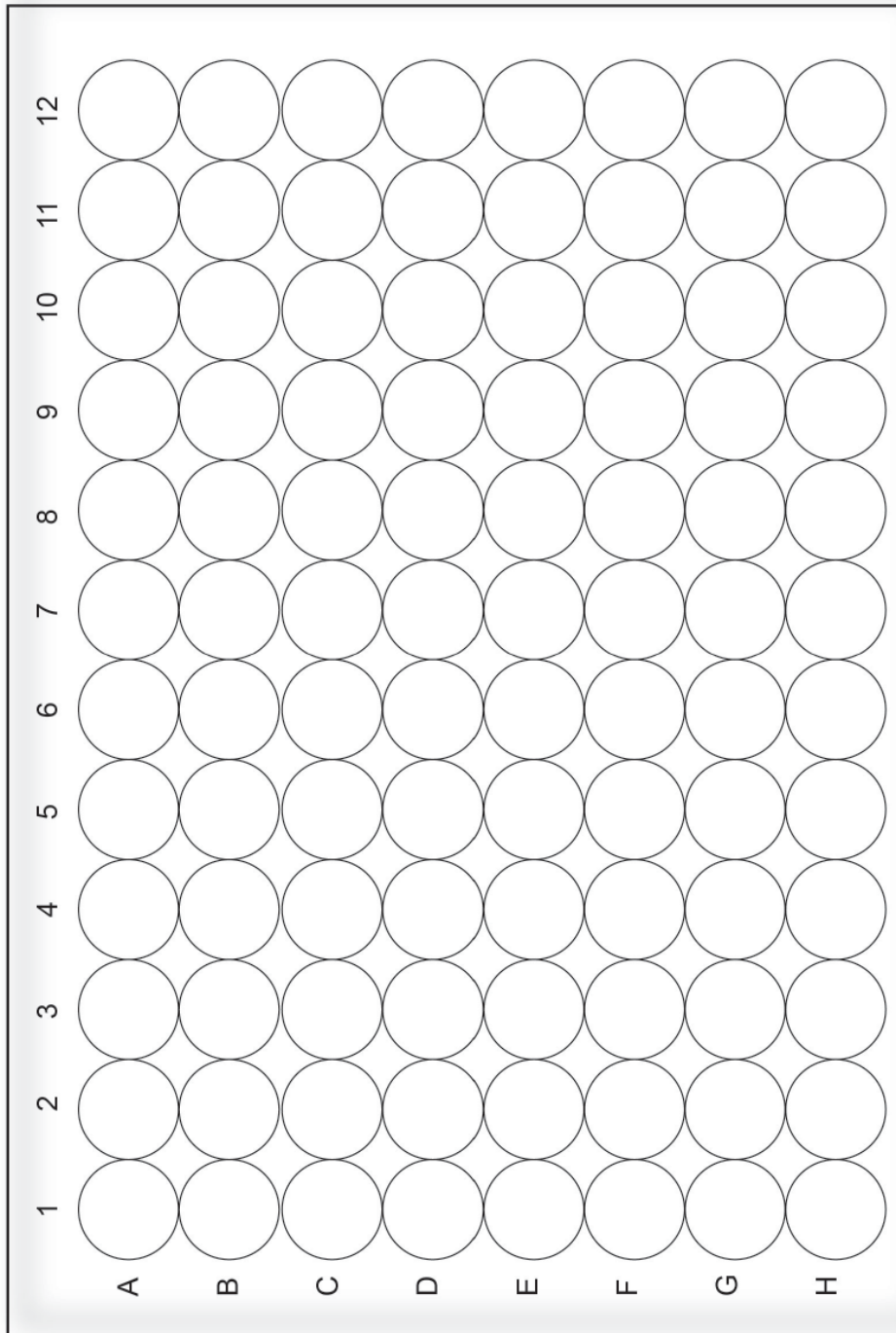
Wavelength: _____

13. **RESULTS/CONCLUSIONS**

_____

_____

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14.   PLATE TEMPLATE

	1	2	3	4	5	6	7	8	9	10	11	12
A	○	○	○	○	○	○	○	○	○	○	○	○
B	○	○	○	○	○	○	○	○	○	○	○	○
C	○	○	○	○	○	○	○	○	○	○	○	○
D	○	○	○	○	○	○	○	○	○	○	○	○
E	○	○	○	○	○	○	○	○	○	○	○	○
F	○	○	○	○	○	○	○	○	○	○	○	○
G	○	○	○	○	○	○	○	○	○	○	○	○
H	○	○	○	○	○	○	○	○	○	○	○	○

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