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SARS-CoV-2 Milk ELISA

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1 Works for me dx.doi.org/10.17504/protocols.io.bk2skyee

XPRIZE Rapid Covid Testing Powell Lab/Lactiga Collab

rlp285

ABSTRACT

Currently, the COVID-19 diagnostics sector includes Point-of-Care and ELISA tests for IgG and IgM that can assess saliva or blood as described by the CDC [Centers for Disease Control and Prevention]. Similarly, the FDA webpage for EUA Authorized Serology Test Performance makes no reference to IgA COVID-19 assays [Food and Drug Administration]. Taken together, there are no direct competitors in the mucosal IgA quantitative diagnostics space.

To address this gap in diagnostic coverage we have generated a novel proof-of-concept dataset on the use of COVID-specific IgA antibodies as a marker of infection. As described below, our assay dataset demonstrates utility across several parameters:
-Highly specific
-Highly sensitive
-Works with undiluted human milk, simplifying sample collection and processing
-Defines a novel correlation between COVID-specific IgA and Secretory Component (SC), where SC is a known surrogate for dimeric IgA, the predominant IgA type in mucosal secretions
-Establishes that dimeric IgA antibodies detected by the assay are capable of neutralizing SARS-CoV-2

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

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PROTOCOL CITATION

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KEYWORDS

SARS-CoV-2, ELISA, milk, IgA, sIgA

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GUIDELINES

keep milk at room temperature for no more than 5 hours, or else keep at 4C for up to 5 days or freeze.

MATERIALS

NAME	CATALOG #	VENDOR
Sulfuric acid	A300C-212	Fisher Scientific
Corning® 96-Well High-Binding Flat-Bottom Microplates 6 Plates/Pack	38019	Stemcell Technologies
Gibco™ (Phosphate Buffered Saline) Solution, pH 7.4 (PBS)	10010-049	Fisher Scientific
Lab Vision™ Tween™ 20 Detergent	cat# TA-125-TW	Thermo Fisher Scientific
BSA	30-AB79	Fitzgerald
Skim Milk Powder		
1-Step™ Ultra TMB-ELISA Substrate Solution	34028	Thermo Fisher
Normal Goat Serum	PCN5000	Thermo Fisher
Falcon 50mL Conical Centrifuge Tubes	14-432-22	Fisher Scientific
MilliporeSigma™ Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody	40-113-52ML	Millipore Sigma
Spike protein	Z03501	Genscript

STEPS MATERIALS

NAME	CATALOG #	VENDOR
Spike protein	Z03501	Genscript
Tween 20	P7949	Sigma Aldrich
PBS pH 7.4		
Normal Goat Serum	PCN5000	Thermo Fisher
Skim Milk Powder		
BSA		Sigma Aldrich
MilliporeSigma™ Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody	40-113-52ML	Millipore Sigma
1-Step™ Ultra TMB-ELISA Substrate Solution	34028	Thermo Fisher
Sulfuric acid	258105	Sigma – Aldrich

MATERIALS TEXT

Reagent or Consumable	Supplier	Catalog #
Text	Text	Numeric
Thermo Scientific 50mL Conical Sterile Polypropylene Centrifuge Tubes	Fisher	12-565-270
Corning™ 96-Well, High Binding, Flat-Bottom, Half-Area Microplate	Fisher	07-200-37
Gibco™ PBS, pH 7.4	Fisher	10-010-023
Tween™ 20, Fisher BioReagents	Fisher	BP337-100
MilliporeSigma™ Calbiochem™BSA, 30% Sterile-Filtered Aqueous Solution, Preservative-Free	Fisher	12-662-650ML
Gibco™ Normal Goat Serum	Fisher	PCN5000
MilliporeSigma™ Calbiochem™ OmniPur™ Nonfat Powdered Milk	Fisher	62-501-0GM
Pierce 1-Step Ultra TMB-ELISA Substrate Solution	Fisher	PI34028

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Sulfuric Acid, Fisher Chemical	Fisher	A510-P500
MilliporeSigma™ Goat anti-Human IgA, alpha-Chain Specific, Peroxidase, Secondary Antibody	Fisher	40-113-52ML
Spike protein (1mg = 1000000ng)	Genscript	Z03501

EQUIPMENT

NAME	CATALOG #	VENDOR
Centrifuge	-	
405 TS Washer	405 TS	Biotek
800 TS Absorbance Reader	800 TS	Biotek

SAFETY WARNINGS

Handle biological samples in a biological containment hood

Milk Processing 1h

1 **00:15:00**

800 x g, 22°C, 00:15:00

Pour fresh milk into 50mL conical tube and centrifuge at 800g for 15min at room temperature

Centrifuge -

2 remove fat using a sterile spatula

3 carefully pour skim milk into a clean 50mL tube

4 repeat centrifugation and fat removal 2x

Day 1 5m

5 Coat half-area 96-well plates with 50ng SARS-CoV-2 spike protein in 50ul PBS [M]**0.001 mg/ml**

50 µl

Spike protein

by Genscript

Catalog #: Z03501

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6 over and store overnight at 4C **Overnight**

4 °C

Day 2 5h

7 Wash plates with 100ul 0.1% Tween 20/PBS (PBS-T) 6x **100 µl**

0.1 % volume

405 TS Washer
plate washer
Biotek **405 TS**

Tween 20
by Sigma Aldrich
Catalog #: P7949

PBS pH 7.4

8 Block plates with 100ul 3% goat serum/0.5% milk powder/3.5% PBS-T for 1h at room temperature **100 µl**

01:00:00

Room temperature

Normal Goat Serum
by Thermo Fisher
Catalog #: PCN5000

Skim Milk Powder
View

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Tween 20
by Sigma Aldrich
Catalog #: P7949

9 Wash as above

10 Add 50ul milk undiluted or diluted with 1%BSA/PBS to each well in duplicate **50 µl**
1 % volume

BSA
by Sigma Aldrich

11 Incubate at room temperature for 2h **02:00:00**
Room temperature

12 Wash as above

13 Add 50ul **50 µl** horseradish peroxidase (HRP)-conjugated secondary antibody: goat anti-human-IgA at 1/3000 in 1%BSA/PBS.

MilliporeSigma™ Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody
by Millipore Sigma
Catalog #: 40-113-52ML

BSA
by Sigma Aldrich

14 Incubate at room temperature for 1h **01:00:00** **Room temperature**

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15 Wash as above

16 Add 50u Pierce 1-Step Ultra TMB-ELISA Substrate Solution

1-Step™ Ultra TMB-ELISA Substrate Solution
by Thermo Fisher
Catalog #: 34028

50 µl

Sulfuric acid
by Sigma – Aldrich
Catalog #: 258105

followed by 50ul 2N hydrochloric acid (HCl)

17 Measure OD at 450nm on an ELISA plate reader

800 TS Absorbance Reader
Plate reader
BioTek 800 TS

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