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SARS-CoV-2 Milk ELISA

DOI

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XPRIZE Rapid Covid Tes...

Powell Lab/Lactiga Collab



rlp285

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Protocol status: Working

We use this protocol and it's working

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Keywords: SARS-CoV-2, ELISA, milk, IgA, sIgA, specific iga antibody, dimeric iga antibody, direct competitors in the mucosal iga, mucosal iga, covid, predominant iga type, elisa, surrogate for dimeric iga, specific iga, undiluted human milk, assay dataset, assay, fda webpage for eua authorized serology test performance, igg, saliva, dimeric iga, igm, eua authorized serology test performance,

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

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

MATERIALS

⊗ Sulfuric acid **Fisher Scientific Catalog #A300C-212**

⊗ Corning® 96-Well High-Binding Flat-Bottom Microplates 6 Plates/Pack **STEMCELL Technologies Inc. Catalog #38019**

⊗ Gibco™ (Phosphate Buffered Saline) Solution, pH 7.4 (PBS) **Fisher Scientific Catalog # 10010-049**

⊗ Lab Vision™ Tween™ 20 Detergent **Thermo Fisher Scientific Catalog #cat# TA-125-TW**

⊗ BSA **Fitzgerald Catalog #30-AB79**

⊗ Skim Milk Powder

⊗ 1-Step™ Ultra TMB-ELISA Substrate Solution **Thermo Fisher Catalog #34028**

⊗ Normal Goat Serum **Thermo Fisher Catalog #PCN5000**

⊗ Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-432-22**

⊗ MilliporeSigma™ Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #40-113-52ML**

⊗ Spike protein **Genscript Catalog #Z03501**

STEP MATERIALS

⊗ Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949**

⊗ PBS pH 7.4

⊗ Normal Goat Serum **Thermo Fisher Catalog #PCN5000**

⊗ Skim Milk Powder

⊗ Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949**

⊗ BSA **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ MilliporeSigma™ Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #40-113-52ML**

⊗ BSA **Merck MilliporeSigma (Sigma-Aldrich)**

⊗ 1-Step™ Ultra TMB-ELISA Substrate Solution **Thermo Fisher Catalog #34028**

⊗ Sulfuric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #258105**

	Reagent or Consumable	Supplier	Catalog #
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	Text	Text	Nu meric
	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	12-662-650 ML
	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
	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



Protocol materials

✕ Skim Milk Powder

✕ Tween 20 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #P7949**

✕ BSA **Merck MilliporeSigma (Sigma-Aldrich)**

✕ 1-Step[®] Ultra TMB-ELISA Substrate Solution **Thermo Fisher Catalog #34028**

✕ Sulfuric acid **Fisher Scientific Catalog #A300C-212**

✕ Spike protein **Genscript Catalog #Z03501**

✕ MilliporeSigma[™] Goat anti-Human IgA alpha-Chain Specific Peroxidase Secondary Antibody **Merck MilliporeSigma (Sigma-Aldrich) Catalog #40-113-52ML**

✕ Normal Goat Serum **Thermo Fisher Catalog #PCN5000**

✕ Falcon 50mL Conical Centrifuge Tubes **Fisher Scientific Catalog #14-432-22**

✕ Corning[®] 96-Well High-Binding Flat-Bottom Microplates 6 Plates/Pack **STEMCELL Technologies Inc. Catalog #38019**

✕ PBS pH 7.4

✕ Gibco[™] (Phosphate Buffered Saline) Solution, pH 7.4 (PBS) **Fisher Scientific Catalog # 10010-049**

✕ Sulfuric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #258105**

✕ Lab Vision[™] Tween[™] 20 Detergent **Thermo Fisher Scientific Catalog #cat# TA-125-TW**

✕ BSA **Fitzgerald Catalog #30-AB79**

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

Equipment

new equipment

NAME

Centrifuge

BRAND

-

SKU



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

0.001 mg/mL

50 µL

 Spike protein **Genscript Catalog #Z03501**



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

[M] 0.1 % volume

Equipment

405 TS Washer

NAME

plate washer

TYPE

Biotek

BRAND

405 TS

SKU

Tween 20 Merck MilliporeSigma (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 Thermo Fisher Catalog #PCN5000

Skim Milk Powder



 Tween 20 **Merck MilliporeSigma (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 μ L

[M] 1 % volume

 BSA **Merck MilliporeSigma (Sigma-Aldrich)**

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

 Room temperature

12 Wash as above

13 Add 50ul  50 μ L 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 **Merck MilliporeSigma (Sigma-Aldrich) Catalog #40-113-52ML**

 BSA **Merck MilliporeSigma (Sigma-Aldrich)**

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

15 Wash as above

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

 1-Step® Ultra TMB-ELISA Substrate Solution **Thermo Fisher Catalog #34028**

 50 μ L

 Sulfuric acid **Merck MilliporeSigma (Sigma-Aldrich) Catalog #258105**

followed by 50ul 2N hydrochloric acid (HCl)

17 Measure OD at 450nm on an ELISA plate reader

Equipment

800 TS Absorbance Reader	NAME
Plate reader	TYPE
BioTek	BRAND
800 TS	SKU