

Materials List for:

Developing a Salivary Antibody Multiplex Immunoassay to Measure Human Exposure to Environmental Pathogens

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URL: <https://www.jove.com/video/54415>

DOI: [doi:10.3791/54415](https://doi.org/10.3791/54415)

Materials

Name	Company	Catalog Number	Comments
Equipment and Software			
Microcentrifuge	Thermo Electron Corporation	75002446	Used to centrifuge samples
Vortex Mixer	VWR	G560	Used to mix samples
Sonicator (mini)	Fisher Scientific	15-337-22	Used to separate beads
Pipettors P10, P20, P100, P1000, 8 ch.	Capp	Various	
Hemocytometer (Bright Line)	Housser Scientific	3200	Used to count coupled beads
Multiscreen Vacuum Manifold	Millipore	MSVMHTS00	Used in washing steps to remove supernatant
MicroShaker	VWR	12620-926	Used to agitate beads during incubations
Tube rack (1.5 ml and 0.5 ml) (assorted)	VWR	30128-346	
Weighing Scale	Mettler or other		Used to measure wash reagents for making buffers
Dynabead Sample Mixer	Invitrogen	947-01	Used during coupling incubation step
MatLab (R2014b)	The MathWorks, Inc.		Used to analyze antibody response data
Microsoft Excel 2014	Microsoft Corporation		Used to analyze antibody response data
Luminex Analyzer with xPonent 3.1 software	Luminex Corporation	LX200-XPON3.1	Instrument and software used to run assay
Antigens			
GI.1 Norwalk Virus: p-particle	Xi Jiang (CCHMC)*	NA	*Cincinnati Childrens' Hospital. Final conc. 5 µg.
GII.4 Norovirus VA387: p-particle	Xi Jiang (CCHMC)*	NA	*Cincinnati Childrens' Hospital. Final conc. 5 µg.
Hepatitis A Virus: grade II concentrate from cell culture	Meridian Life Sciences	8505	Antigen coupled at 100 µg
<i>Helicobacter pylori</i> : lysate	Meridian Life Sciences	R14101	Antigen coupled at 25 µg
<i>Toxoplasma gondii</i> : recombinant p30 (SAG1)	Meridian Life Sciences	R18426	Antigen coupled at 25 µg
<i>Campylobacter jejuni</i> : heat killed whole cells	KPL	50-92-93	Antigen coupled at 50 µg

Primary Antibodies			
Guinea pig anti-Norovirus	(CCHMC)*	NA	Used for coupling confirmation
Mouse anti-Hepatitis A IgG	Meridian Life Sciences	C65885M	Used for coupling confirmation
Mouse anti-Hepatitis A IgG	Meridian Life Sciences	C65885M	Used for coupling confirmation
BacTraceAffinity Purified Antibody to <i>Helicobacter pylori</i>	KPL	01-93-94	Used for coupling confirmation
Goat pAb to <i>Toxoplasma gondii</i>	Abcam	Ab23507	Used for coupling confirmation
BacTrace Goat anti- <i>Campylobacter</i> species	KPL	01-92-93	Used for coupling confirmation
Secondary Antibodies			
Biotin-SP-Conjugated AffiniPure Donkey anti-Goat IgG (H+L)	Jackson	705-065-149	Used for coupling confirmation
Biotinylated Rabbit anti-Goat IgG (H+L)	KPL	16-13-06	Used for coupling confirmation
Biotinylated Goat anti-Mouse IgG (H+L)	KPL	16-18-06	Used for coupling confirmation
Affinity Purified Antibody Biotin Labeled Goat anti-Rabbit IgG (H+L)	KPL	176-1506	Used for coupling confirmation
Consumables			
1.5 ml copolymer microcentrifuge tubes	USA Scientific	1415-2500	Used as low binding microcentrifuge tubes
10 µl pipette tip refills	BioVentures	5030050C	
200 µl pipette tip refills	BioVentures	5030080C	
1,000 µl pipette tip refills	BioVentures	5130140C	
Aluminum foil	Various Vendors		Used keep beads in the dark during incubations
Deep Well plates	VWR	40002-009	Used for diluting saliva samples
Multiscreen Filter Plates	Millipore	MABVN1250	Used to run assays
Oracol saliva collection system	Malvern Medical Developments Limited		Used for saliva collection
Reagents			
Carboxylated microspheres (beads)	Luminex Corporation	Dependent on bead set	Antigens are coupled to the microspheres
EDC (1-ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride)	Pierce	77149 or 22980	Used in bead activation
Sulfo-NHS (N-hydroxysulfosuccinimide)	Pierce	24510	Used in bead activation
Streptavidin-R-phycoerythrin (1 mg/ml)	Molecular Probes	S-866	Used as reporter
MES (2-[N-Morpholino]ethanesulfonic acid)	Sigma	M-2933	Used for coupling
Tween-20 (Polyoxyethylenesorbitan monolaurate)	Sigma	P-9416	Used in wash buffer to remove non-specific binding
Protein Buffers			
PBS-TBN Blocking/ Storage Buffer (PBS, 0.1% BSA, 0.02% Tween-20, 0.05% Azide, pH 7.4)**			Filter Sterilize and store at 4 °C
PBS, pH 7.4	Sigma	P-3813	138 mM NaCl, 2.7 mM KCl
BSA	Sigma	A-7888	0.1% (w/v)

Tween-20	Sigma	P-9416	0.2% (v/v)
Sodium Azide (0.05% azide)**	Sigma	S-8032	**Caution: Sodium azide is acutely toxic. Avoid contact with skin and eyes. Wear appropriate PPE's. Dispose of according to applicable laws.
MES/Coupling Buffer (0.05 M MES, pH 5.0)			
MES (2-[N-Morpholino]ethanesulfonic acid)	Sigma	S-3139	
5 N NaOH	Fisher	SS256-500	
Assay Buffer (PBS, 1% BSA, pH 7.4)			Filter Sterilize and store at 4 °C
PBS, 1% BSA, pH 7.4	Sigma	P-3688	138 mM NaCl, 2.7 mM KCl, 1% BSA
Activation Buffer (0.1 M NaH₂PO₄, pH 6.2)			Filter Sterilize and store at 4 °C
NaH ₂ PO ₄ (Sodium phosphate, monobasic anhydrous)	Sigma	S-3139	0.1 M NaH ₂ PO ₄
5 N NaOH	Fisher	SS256-500	
Wash Buffer (PBS, 0.05% Tween-20, pH 7.4)			Filter Sterilize and store at 4 °C
PBS, 0.05% Tween-20, pH 7.4	Sigma	P-3563	138 mM NaCl, 2.7 mM KCl, 0.05% TWEEN