ZIVA™ Assays with AIR™ Technology

Human Acute Respiratory Virus Serology Array V1.0

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For Research Use Only. Not for Use in Diagnostic Procedures.
For any questions, contact our Support Team at 314-925-7800.

Introduction

The Acute Respiratory Viral Serology (ARVS) Array is a multiplex assay for high-throughput, high-definition serological studies of 27 unique validated recombinant antigens interrogating 3 different viruses. These viruses and their subtypes include Coronaviruses (SARS-CoV-2, SARS-CoV, MERS-CoV, and 4 common-cold Coronaviruses), Influenza A (H3N2, H1N1, H7N9, H5N1), Influenza B (3 lineages), and Respiratory Syncytial Viruses A and B. The Coronaviruses have higher definition to interrogate the Spike Receptor Binding Domain (RBD), Spike S1, Spike S1 +S2, and Nucleocapsid proteins (varies by Coronavirus subtype, see Table 2). SARS-CoV-2 also includes the mutant D614G protein. The array is designed to determine the antibody profile in serum samples with applications in epidemiology and serosurveillance, vaccine development, and basic translational research.

Adarza® Platform

Arrayed Imaging Reflectometry (AIR™) is a silicon chip-based label-free biosensor platform. It optically senses molecular binding at the biosensor surface by enabling the detection of any probe or target pair of analytes.

By functionalizing the surface with highly specific probe molecules (eg. antibodies, protein kinases, microRNAs, etc.) target binding can be detected and quantified directly with high sensitivity. As a label-free technique, the assay procedure requires minimal process time. Comparable labeled techniques require two or three additional chemistry steps after target binding, typically resulting in process times greater than two hours and adding substantial reagent cost.

The low complexity of Adarza's AIR™ method enables massive multiplexing, allowing hundreds and potentially thousands of unique probes to be arrayed on the chip surface for simultaneous detection without interference.

The flexibility of AIR™ enables for the first time the same technology platform to be used across the entire biomarker discovery and validation process through deployment in clinical and point of care diagnostics applications.

The ZIVA™ platform and kits allow for greater sensitivity, the ability to detect not only the *low* level analytes, but also the *high* level in the **same** assay. ZIVA™ reads your samples fast with a high level of accuracy and minimal procedural steps—keeping the process simple, accurate, and reproducible.

Each kit includes:

-Positive Controls to ensure optimal results

-Plate of Biosensors utilizing cutting edge technology to get you consistent, sensitive results

-Fully tested and high functioning reagents for your analytes of interest

This guide will provide detailed instructions for the preparation of assay reagents for your ZIVA™ Multiplex Immunoassay Kit. For additional instrument protocols, please refer to your instrument guide. SDS are available upon request and proper PPE is needed during execution.

Safety Note:

Proper PPE (gloves, lab goggles/glasses, and lab coat) is needed when performing kit assay. For full SDS documents, please see the Adarza® website. www.adarzabiosystems.com

All materials must be disposed of as determined by your institution, state and/or federal guidelines.

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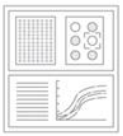
Please read entire protocol before use.

ADARZADARZADARZA®
BIOSYSTEMS

The Process

Simple Work-Flow and Rapid Turn-Around

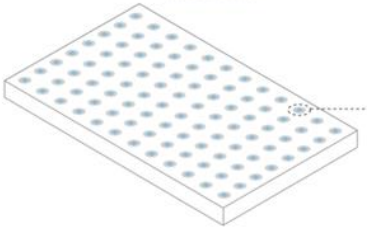


Apply Sample to Chip & Incubate
Manual to fully automated

Rinse, Dry, Acquire Image
Fully automated walk-away operation

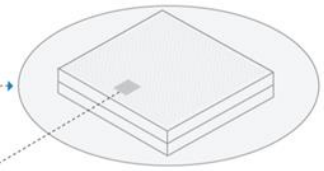
Analyze Data
Image to first results in minutes

96 Well Plate



Each 96 well plate tests up to 400 analytes per well

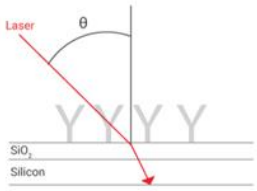
Singular Well

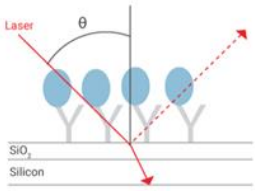


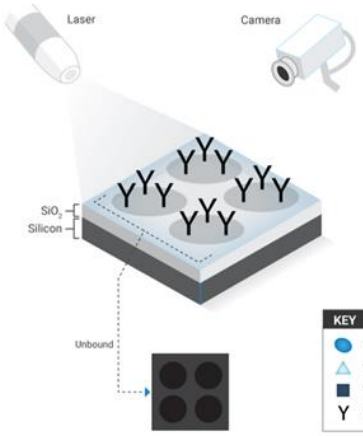
Up to 400 unique analytes detected over thousands of spots in each well

How it Works

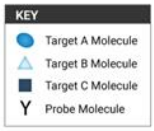
A laser beam is reflected off the chip surface and imaged with a digital camera in a fraction of a second, producing an array of bright and dark spots.

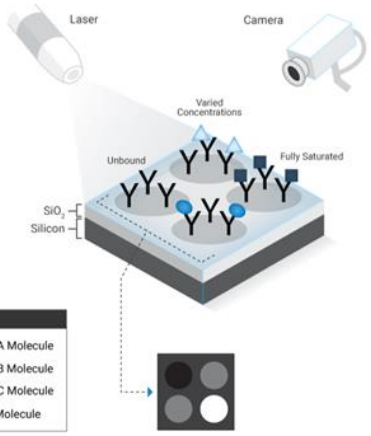




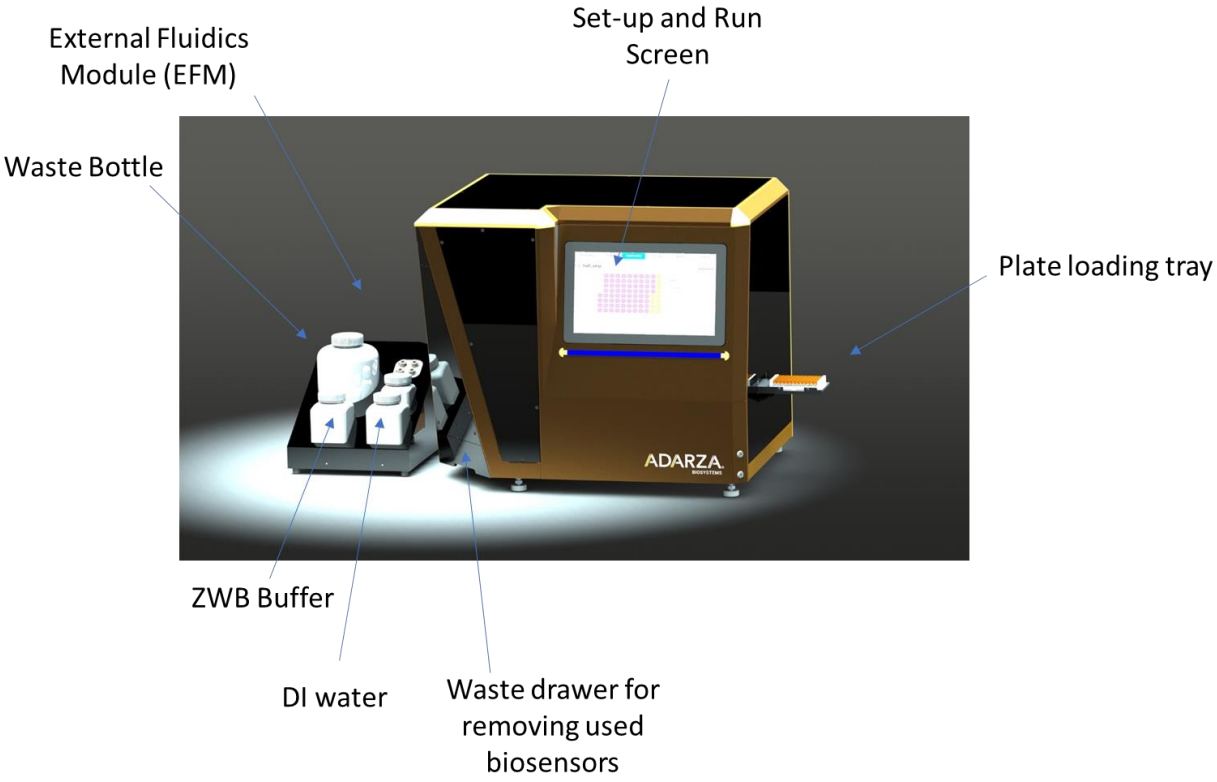


Perfect thickness, no reflection





Thicker surface, some light reflects


External Fluidics Module (EFM)
Set-up and Run Screen
Waste Bottle
Plate loading tray
ADARZA
ZWB Buffer
DI water
Waste drawer for removing used biosensors

Kit Contents and Storage

Reagents Supplied

Assays are offered in a convenient kit format that includes necessary buffers, reagents, and diluent components in a single box (Table 1). Additional water and samples are necessary for full evaluation. Please perform all instrument setup steps before using ZIVA™. A full list of instructions can be found in the instrument guide and a quick summary is found within this document.

Table 1. ZIVA™ Kit Box Contents

Reagent	Item Number	Volume	Quantity	Storage
96 well consumable with biosensor array		N/A	1	4°C
Clear Plate Seal		1	1	RT
Plate Lid		1	1	RT
Negative Control		100µl	1	4°C
Positive Control*		25µl	1	4°C
Assay Buffer		16ml	1	4°C
ZIVA™ Wash Buffer (ZWB)		50ml of 20X	1	4°C

***The positive control contains a mixture of antibodies that result in a positive response to the following antigens:**

SARS-CoV-2 RBD, SARs-CoV-2 S1, SARS-CoV-2 D614G, SARS-CoV RBD, MERS-CoV RBD, Influenza B Malaysia, Influenza B Phuket

Table 2: Biosensor Array Targets

Acute Respiratory Viral Serology Array-27 Plex	
Influenza A and B 9 unique targets	Influenza A H3N2 (2 origins: Wisconsin and Texas)
	Influenza A H1N1 (2 origin: California and Beijing)
	Influenza A H7N9
	Influenza A H5N1
	Influenza B (3 lineages: Massachusetts, Phuket, Malaysia)
RSV 2 types	RSV A Glycoprotein
	RSV B Glycoprotein
Common Coronavirus 4 types	HCoV-HKU1
	HCoV-229E
	HCoV-OC43
	HCoV-NL63
SARS-CoV-2 (COVID-19) 6 antigen targets	Spike S1
	Receptor Binding Domain (RBD)
	Nucleocapsid
	Spike S1+S2 ECD
	Spike S2 ECD
	Spike S1 D614G mutant variant
MERS CoV 3 antigen targets	Spike S1
	Receptor Binding Domain (RBD)
	Nucleocapsid
SARS-CoV 3 antigen targets	Spike S1
	Receptor Binding Domain (RBD)
	Nucleocapsid

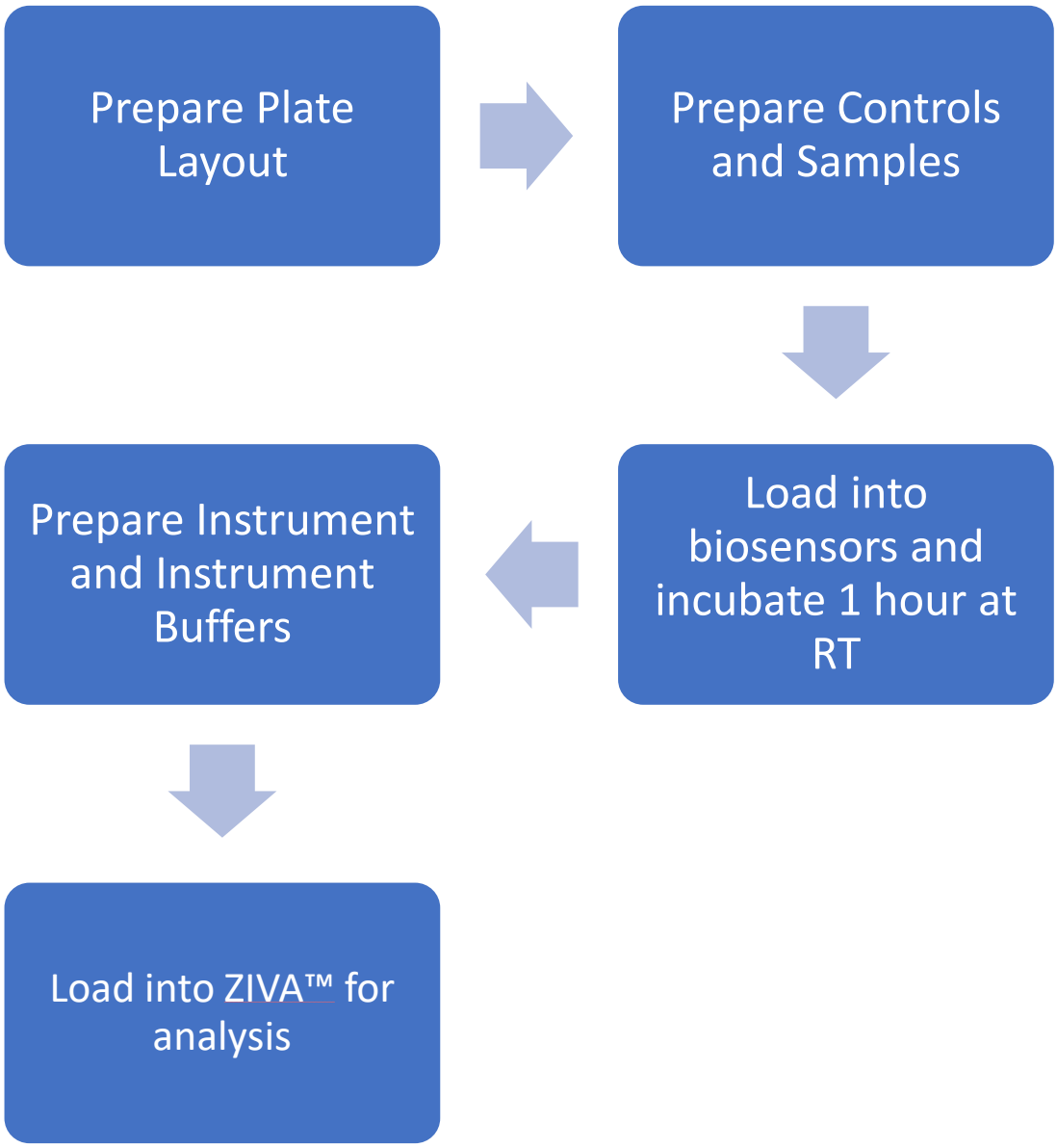
Table 3: Additional Materials/Recommended Materials Needed

Reagent/Material
Multi-channel Pipettors (8-12 channel 200µl capacity)
Single channel Pipettors (10, 200µl and 1000µl)
Low Binding Pipette tips (range of 10-1000µl)
RO/DI H_2O 1000 mL
Vortex Mixer
Low Binding Microcentrifuge tubes
Tube Rack
Reagent Reservoirs

Storage and Stability

Upon receiving your kit, the box can be stored in 4°C until use. All components are guaranteed for a minimum of 3 months from date of purchase when stored as specified.

Protocol Quickview:



Protocol

Assay Reagent and Sample Preparation

Remove Reagents and place on ice:

- Negative and Positive Controls
- Assay Buffer

Sample Preparation

The sample collection and storage conditions listed below are intended as general guidelines.

Serum: Use a serum separator tube (SST) and allow samples to clot for 30 minutes at room temperature before centrifugation for 15 minutes at 1000 x g. Remove serum and assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Plasma: Collect plasma using heparin or EDTA as an anticoagulant. Centrifuge for 15 minutes at 1000 x g within 30 minutes of collection. Assay immediately or aliquot and store samples at ≤ -20° C. Avoid repeated freeze-thaw cycles.

Frozen samples (serum or plasma): When using frozen samples, it is recommended to thaw the samples completely, mix well by vortexing and centrifuge prior to use in the assay to remove particulates.

Sample Dilution for Assay: Dilute samples **1:20** in Assay Buffer. It is recommended to run samples in duplicate. Label sample tubes and add 114µL of Assay Buffer to each tube. After spinning samples, add 6µL of each sample into the appropriate tube and vortex gently to mix. (It may be necessary to dilute further or less, but this will be dependent on concentration of sample). You will be loading 50 µl of sample/buffer mix to each duplicate well.

Negative and Positive Controls

A. Vortex both Controls to ensure they are fully suspended.
B. Label 2 tubes “Negative Control” and “Positive Control”. To each tube add 114µl of Assay Buffer and then add 6µl of the Positive or Negative Control to the appropriate tube.

Assay and Plate Preparation

A. **Prepare Plate Template/Map**

ZIVA™ allows 3 options to configure a plate layout, review instrument guide for further instructions:

Upload a default recommended layout

OR

Generate a layout with the template generator (you can edit sample names and move around placement of controls)

OR

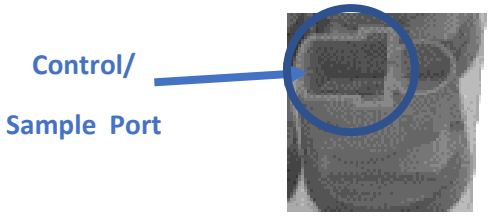
Upload a CSV file to apply a plate template
(built on your computer and specific to your samples-Great for High Throughput!)

B. **Place Strips into Metal Plate**

When the strips are received, they will be sitting within a holding rack for shipping. The strips will need to be loaded into the metal loading plate that was included with ZIVA™.

Tip: Take care to ensure strips are seated fully and surface is flat within metal tray. Push strips down when tray is seated on the lab bench, NOT in ZIVA.

1. Transfer 50µL of Diluted, Prepared Samples and 50µl of Negative and Positive Controls to the *Control/Sample port* of the respective wells of the assay plate according to plate layout. Take care to avoid any sample addition or splashing into the enhancer port.

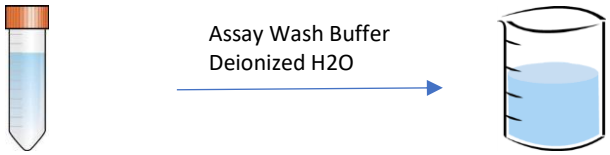


2. Gently tap the assay plate on the benchtop to move diluents into assay plate, and begin reaction
    - You will see a color change to dark blue across plate. This shows diluents have reached and saturated the chip.
3. Let plate sit for 5 minutes at RT before adding seal.
4. Gently press plate seal over plate taking care to ensure each well is covered.
5. Incubate assay plate 1 hour at RT.

Instrument Reagent Preparation (perform during incubation time)

A. **ZIVA™ Wash Buffer (ZWB)**

1. Combine 50mL of 20X ZWB into empty wash buffer bottle. Fill to 1L fill line on bottle. Invert gently 3-5 times to ensure full mixing. You now have 1X ZWB.

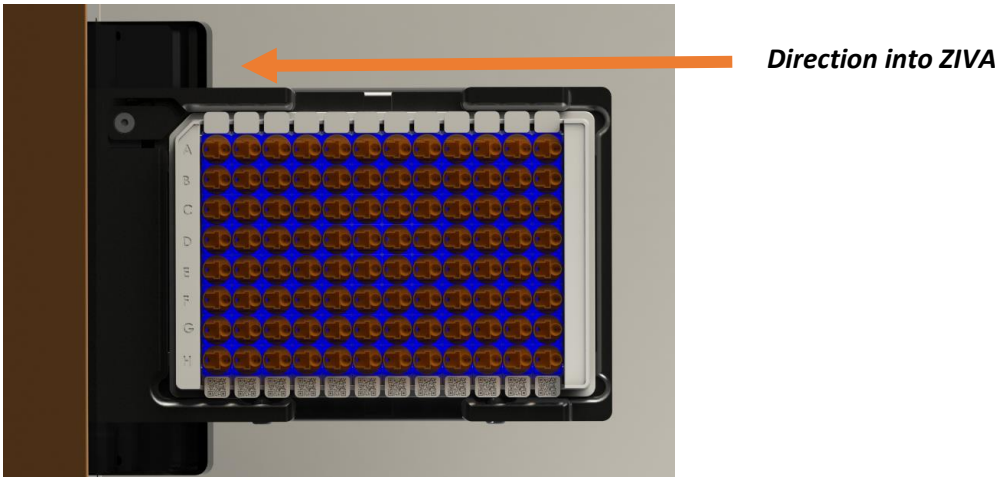


2. Apply EFM Cap with quick release fittings and place bottle back into the EFM (For additional information, see instrument guide).
3. Fill DI water container to fill line. Reconnect fluid lines to bottle in the EFM on the side of the instrument.
4. Click "Wake" to initiate priming of lines with buffer before running the instrument.
5. Dispose of any biosensors in the waste bin before beginning the run.

Tip: ZIVA will also give a check list before the plate run begins.

Final Reaction Run and Analysis

6. After incubation, remove plate seal and lid and insert plate into instrument with notched A1 corner going in first on side furthest from the user. Barcodes should be on edge closest to the user. See picture below for further details.



7. **Start ZIVA™**
8. Select Plate layout and click “Start Run” and ZIVA™ will begin processing the plate.*
9. Data can then be analyzed and exported from your desktop via the **ZIVA™ Dashboard.**

**For more details on ZIVA™ and setup of the Instrument, please review the Instrument Quick Guide*

Post ZIVA™ Run/End of Day Shutdown

A. When the run is complete, discard the strips used in the plate and ensure waste drawer is empty.
    1. Click Sleep on ZIVA™ main screen to initiate cleaning of wash lines. Ensure water bottle is filled with water before initiating.
    2. See instrument guide for detailed instructions.
B. Once ZIVA reaches sleep state, empty waste fluids and dispose of them properly. Rinse out waste containers with water.
    1. Dispose of waste according to institution guidelines. For full SDS information or questions, contact our customer support team at 314-925-7740.

For long term shutdown and maintenance care of ZIVA™, lines and waste containers should be rinsed and primed with 5-10% ethanol solution. Instrument can be shut down for prolonged periods of non-use. For full details on long term shutdown, instrument error codes, and other instrument questions, please check out our full ZIVA™ instrument guide.

Table 4: Full Labels of Hazardous Components

Kit Component	Catalog #	Full Label	Details
Positive Control			Contains mild irritants and products of animal origin.
Negative Control			Contains products of animal origin.
Assay Buffer			Contains mild irritants and products of animal origin.
20x ZIVA™ Wash			Irritant

Example Plate Template

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