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Detect^{X}Rv Kit

bkatchman [1]

[1]PathogenDx

1 Works for me dx.doi.org/10.17504/protocols.io.bkg4ktyw

elachance

ABSTRACT

The Detect^{X}-Rvtest is a test based on end-point reverse transcription polymerase chain reaction (RT-PCR) coupled to DNA microarray hybridization for the detection of multiple genes within SARS-CoV-1 and SARS-CoV-2 viruses.The DNA microarray contains:

- Detect^{X}-Rv Kit –

-Detect^{X}-Rv SARS-CoV-2 Multiplex Assay—contains 5 (five) SARS-CoV-2 primer sets; 4 (four) SARS-CoV-2probes targeting each N1, N2, and N3 genes

-Detect^{X}-Rv RNase P Control—internal process control for nucleic acid extraction 2 (two) RNase P primer and probe sets as an internal positive control.

- Detect^{X}-Rv SARS-CoV-2 Control – RNA control that contains targets specific to the SARS-CoV-2 genomic regions that are targeted by the assay

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STEPS MATERIALS

NAME	CATALOG #	VENDOR
Quick-DNA/RNA Viral MagBead	R2140 / R2141	Zymo Research
SSC (20X), RNase-free	AM9770	Thermo Fisher
Denhardt's Solution (50X)	750018	Thermo Fisher
AccessQuick(TM) RT-PCR System, 100 reactions	A1702	Promega

EQUIPMENT

NAME	CATALOG #	VENDOR
Plate Fuge	C2000	
Mini Amp	A37834	Applied Biosystems

NAME	CATALOG #	VENDOR
Hybridization Chamber	HYB-001	
Analogy Vortex Mixer	02-215-414	Fisher Scientific
ZR-96 MagStand	P1005	Zymo Research
Sensospot	SensoSpot	Sensovation

DISCLAIMER:

RNA Extraction 1h 30m

1 - Extract RNA with the Zymo Research Quick-DNA/RNA Viral MagBead Kit (R2140 or R2141): For a complete description view the Zymo Research Quick-DNA/RNA Viral MagBead Kit product insert. Follow the manufacturer's guidelines for the proper use and procedure for this product.

Quick-DNA/RNA Viral MagBead
by Zymo Research
Catalog #: R2140 / R2141

ZR-96 MagStand
Magnetic Bead
Zymo Research **P1005**

2 - Place the nasopharyngeal swab into 400 µL – 2mL 1x VTM for storage prior to RNA extraction.

3 - If using a swab transported in VTM, vortex for 10 seconds to release viral particles and remove 400 µL for Sample Preparation and DNA/RNA Purification procedures per the Zymo Research Quick-DNA/RNA Viral MagBead Kit. 1h 30m

Analogy Vortex Mixer
Fisherbrand **02-215-414**

- Store any remaining samples at -20°C for up to two weeks or -80°C for long term storage.

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5 - Once complete the extracted RNA can be used immediately or stored frozen at -20°C for up to two weeks or -80°C for long term storage.

One Step RT PCR 2h

6 Mix and briefly centrifuge each component before use. Combine the following into a master mix, multiply per reaction as shown in Table 1:

AccessQuick(TM) RT-PCR System, 100 reactions
by Promega
Catalog #: A1702

(Volume per reaction) - Component - Final Concentration

25 µl - Access Quick Mastermix, 2X - 1X

2 µl - RT-PCR Primer Set 1- [M]**1 Micromolar (µM)**

1 µl - AMV RT (5u/microliter) - 0.1u/microliter

5 µl - Purified RNA Sample - N/A

17 µl - Nuclease Free Water

50 µl Total Volume per Reaction

7 - Determine the number of samples in the reaction and multiply each reagent to prepare the master mix, leaving out the Purified RNA Template from the master mix and add individually to each designated well

8 - Mix by pipetting and add 45 µl of the master mix per well

9 - Add 5 µl of the RNA template to each designated well making sure to change tips between samples

10 Cover the plate with clear adhesive film and seal.

11 Centrifuge the plate in a for 30 seconds.

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Plate Fuge
PCR Plate Spinner
Benchmark C2000

12 Place the plate in the thermal cycler and cover with a heat pad before closing the thermal cycler cover.

Mini Amp
Thermal Cycler
Applied Biosystems A37834

13 Input the Reverse Transcriptase and PCR cycling program as shown in Table

Step	Temperature (oC)	Time	Cycle Number	
First Strand cDNA Synthesis	Reverse Transcription	45 min	1x	
AMV RT Inactivation and RNA/cDNA/primer denaturation	94	2 min	1x	
PCR Amplification				
Denature	94	30 sec	40x	
Annealing	55	30 sec		
Extension	68	30 sec		
Final Extension	68	7 min	1x	

DNA Hybridization

14 General guidelines to follow for hybridization: When pipetting with the multichannel onto the microarray slide, only dispense to the first stop. DO NOT depress the multichannel to the second stop, or full evacuation of the tips to avoid cross contamination.

15 Before starting, thaw Buffer 2 at room temperature. You will need Buffer 1 as well

SSC (20X), RNase-free
by Thermo Fisher
Catalog #: AM9770

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Denhardt's Solution (50X)
by Thermo Fisher
Catalog #: 750018

16 Cut paper towel to size to fit the bottom of the hybridization chamber provided.

Hybridization Chamber
PathogenDx **HYB-001**

17 Place the plate to be used in the Hybridization Chamber.

18 Apply 200 µL of Molecular Biology Grade Water to each well of the 96-well while being careful to avoid contact with the array.

19 Aspirate and then again, dispense 200 µL of Molecular Biology Grade Water to each well of the 96-well and allow to sit covered in the Hybridization Chamber for 5 minutes before aspirating water from the slides.

20 Prepare the Pre-hybridization Buffer and Hybridization Buffers in clean tubes for the number of microarrays that will be hybridized as per Tables 5 and 6. Vortex briefly to mix.

20.1 Table 5: Reagent Volumes for preparation of PRE-

Analogy Vortex Mixer
Fisherbrand **02-215-414**

Hybridization Buffer

96-Well Plate	**Volumes corresponding to a full 96 well plate being hybridized**
Molecular biology grade water (µL)	16761
Buffer 1 (µL)	4976
Buffer 2 (µL)	2618

Calculations include 20% extra volume for pippetting errors

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20.2 Table 6: Reagent Volumes for preparation of Hybridization Buffer

96-Well Plate	**Volumes corresponding to a full 96 well plate being hybridized**
Buffer 1 (µL)	1390
Buffer 2 (µL)	700

Calculations include 20% extra volume for pippetting errors

21 Aspirate the water wash and add 200 µL of Pre-hybridization Buffer to each well of the 96-well slides without touching the pipette tip to the array surface. Close the Hybridization Chamber box lid.

22 Allow Pre-hybridization Buffer to stay on the arrays for 5 minutes; do not remove slides from the Hybridization Chamber.

23 Briefly centrifuge the tubes or plate containing the Labeling PCR product.

24 Add 18µL of Hybridization Buffer to each well of the Labeling PCR product within the 96-well PCR plate or tubes, pipette up and down to mix. It is important that no cross- contamination occurs during this step. The PCR product and the Hybridization Buffer mix constitute the Hybridization Cocktail.

25 Aspirate Pre-hybridization Buffer from the arrays. **Caution:** ***Do not allow the arrays to air dry. Avoid contact with the array surface***

26 Immediately add 68µL (68 µL – Total Volume of PCR Reaction + Hyb Buffer) of the Hybridization Cocktail to each array of the 96-well being careful not to touch the array surface with the pipette tip. Ensure that the sample ID and location are recorded.

27 Close the Hybridization Chamber lid.

28 Allow to hybridize for 30 minutes at room temperature in the Hybridization Chamber. **Caution:*****Avoid moving the Hybridization Chamber while hybridizing to avoid sample merging.***

Post Hybridization Plate Processing

29 Prepare Wash Buffer according to the number of slides to be used. (Table 7). Washing must be performed according to the protocol to ensure detectable signal and adequate washing to prevent elevated backgroundsignals.

29.1 Table 7

	Volumes corresponding to a full 96 well plate being hybridized
Buffer 1 **(µL)**	**777.6 µL**

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Molecular biology grade water ***(mL)****	**102.9 *mL***
Total volume (mL)*	**103.7 *mL***

*Volumes are measured in mL
*** Calculations include 20% extra volume to account for pipetting errors.*

30 Aspirate Hybridization Cocktail from the plates

31 The washing steps below (32-37) must occur quickly to ensure no drying occurs

32 Add 200 µL of Wash Buffer to each well of the 96-well, mix by pipetting 5 times, then aspirate.

33 Add 200 µL of Wash Buffer to each well of the 96-well, mix by pipetting 5 times, then aspirate.

34 Add 200 µL of Wash Buffer to each well of the 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 5 minutes, aspirate

35 Add 200 µL of Wash Buffer to each well of the 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 5 minutes, aspirate

36 Add 200 µL of Wash Buffer to each well of the 96-well, mix by pipetting 5 times, allow buffer to remain on the slides for 5 minutes, aspirate

37 Perform a final wash by dispensing and aspirating 200 µL of Wash Buffer 2 times to each well of the 96-well.

38 Following the last aspiration step, remove the slides from the Hybridization Chamber. *Do not allow drying to occur on slide surface. Therefore, if running more than plates, aspirate the two and move those to Step 37 - allow the final to dispense of Wash Buffer to remain on the additional slides until the Slide Spinner is available for use. This will ensure no drying occurs on the slide surface.*

39 Load the slides into the plate centrifuge for 1 minute to completely dry the wells in the plate

40 PathogenDx plates should be placed back into a moisture barrier bag with desiccant until scanning may be performed to protect the slides from light. Slides should be scanned within two weeks of hybridization.

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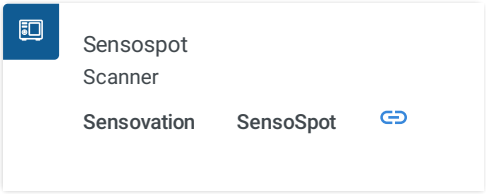
Scanning and Data Acquisition

41 Access the Sensovation scanner desktop, select the application “Array Reader”.



42 Open the tray, select “Open Tray”.

43 Place the slides in the tray oriented with the barcode towards the technician and face down. a. If scanning a 96-well plate, place the plate face up and aligned with A1 in the top left

44 Close the tray, select “Close Tray”.

45 Select “Scan”.

46 From the dropdown menu select the 12-well slide – PDx, 96-well – PDx.

47 Note: All other information on this screen is preprogrammed – do not alter.

48 Select the Blue Arrow to begin the scanning process.

49 While the slides are being scanned, select “Result Overview” to review the images of the wells.

50 When the slides are finished scanning and the screen displays the digital image of a slide with all green wells, select the Red X to exit the scanning process.

51 Open the tray, select “Open Tray”.

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52 Remove the slides and store in the slide case inside the moisture barrier bag with the desiccant packets.

53 Close the tray, select “Close Tray”.

54 Exit the Array Reader application, select “Exit”.

55 On the Sensovation Scanner desktop, select the folder “Scan Results”.

56 Locate the folder associated with your slide and rename the folder with the slide barcode number by scanning the barcode located either on the outside of the barrier bag or on the slide itself. (ex. rename: ScanJob-191108130334_1 to 7024001001)

57 Submit the whole barcode labeled folder to the “Image Folder” within Dropbox.

58 The folder will automatically begin uploading, the PathogenDx Augury© Software will analyze the data and directly deposit the reports into the “Reports” folder within Dropbox.

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