Detection of Viruses

The VirWaTest Detection Method allows the detection of Human Adenovirus and/or Hepatitis E Virus present in water samples through a Quantitative PCR assay.

The detection is carried out by adding a certain amount of the eluate resulting from the VirWaTest Extraction Method into microtubes containing a small white bead composed of the components needed for the assay, which is performed by using a thermocycler connected to a computer. When the assay is finished the software provided with the thermocycler allows to determine if the water sample was positive for the presence of these viruses.

The reagents may be used with any quantitative PCR equipment. In this protocol, the procedure described uses a Mini8 Plus Real-Time PCR from Coyote Biosciences that is a thermocycler coupled to a battery. You may contact us at www.virwatetest.org for further assistance.

Kit Contents

Reagents

| Human Adenovirus PCR Microtubes, 4 eight-tube Units | Hepatitis E Virus PCR Microtubes, 4 eight-tube units | Water, 2 mL x 1 Unit |

Equipment and Reagents

| Micropipette, 1 Unit | Micropipette Tips, 1 Box |

| Marker, 1 Unit |

Material Not Included

| Quantitative PCR Thermocycler | Power Adapter |
Micropipette Operation

The micropipette supplied with the kit allows to pipet volumes from X microlitres to X microlitres. Follow this procedure to pipet the needed volumes:

A. Hold the micropipette horizontally and locate the plastic window where the numbers are displayed.

B. Make sure the lock lever is in the Unlock position. While the lock lever is in the Unlock position you should be able to roll the plunger easily. If it is not, turn it to the Unlock position.

C. Roll the plunger to adjust the volume indicator to the desired volume. Never take the numbers out of the volume range the micropipette is able to handle.

D. Turn the lock lever to the Locked position to prevent the plunger from rolling inadvertently.
E. Place a tip at the end of the micropipette. Do not touch tips with bare hands, push the micropipette against the tip instead.

F. Before immersing the tip into the solution push the plunger and hold it down. Then immerse the tip into the solution.

Micropipette plungers have two pushing-positions in form of two points of resistance. Before loading the pertinent volume push until the first point of resistance. When delivering the loaded volume push all the way to the bottom.

G. While the tip is sunken into the solution release the plunger gently to draw up the volume.
H. Move the micropipette to the tube you want the volume to be dispensed.

I. Push the plunger down to dispense the volume.

J. Push down the tip-eject button to discard the tip.

Assay Preparation

1. The microtubes specific for the detection of Human Adenovirus and of Hepatitis E Virus are provided in two separate boxes. Use the appropriate microtube strip depending on the virus that is aimed to be detected.

2. Be sure that the white beads are at the bottom of the tubes. If they are not, gently tap the tube with the finger until the bead goes to the bottom.

3. Add 17.5 microlitres of water to each microtube by using the micropipette.
4. Add the sample to the microtube by using the micropipette. Also add water to one tube as a negative control. The corresponding volumes to be added are the following:

- **10 microlitres of sample product when detecting Adenovirus.**
- **5 microlitres of sample product when detecting Hepatitis E.**
- Nuclease-free water as a negative control:
  - **10 microlitres for Adenovirus detection assays.**
  - **5 microlitres for Hepatitis E detection assays.**

5. Mix the solution by inverting the microtube until the bead gets dissolved. The reaction is now ready for the detection step.

6. Place the tube-strip in the thermal plate of the thermocycler.
**Quantitative PCR Assay Setup**

1. Plug the Mini8 Plus Real-Time PCR cycler both to the power and to the computer by using the power adapter and the USB cable supplied with the device.

2. Turn on the computer and run the Mini8 Plus Real-Time PCR System software. When asked about allowing the application to make changes on the computer, click on Yes.

3. Go to the **Setup** section to set up the PCR thermal profile and the plate layout.

4. Click on the **New Experiment** button. Fill the gaps with the experiment name and the sample type and click OK.

5. To set up the thermal profile corresponding to the virus that is aiming to be detected select the **Thermal Protocol** tab and click on **Select Protocol**. Then select the file (Thermal Profile.pdt) and click on **Open File**.

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Time</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>95°C</td>
<td>10 minutes</td>
<td>1</td>
</tr>
<tr>
<td>95°C</td>
<td>15 seconds</td>
<td>40</td>
</tr>
<tr>
<td>60°C</td>
<td>1 minute</td>
<td></td>
</tr>
</tbody>
</table>

6. To set up the reaction plate select the **Plate Setup** tab and click on **Select Plate**. Then select the plate configuration file (Plate Configuration.pse) and click **Open File**.
Note: By default, the plate configuration file is set up according to the following tube distribution:

<table>
<thead>
<tr>
<th>Microtube</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample A</td>
<td>Sample A 1:10 Dilution</td>
<td>Sample B 1:10 Dilution</td>
<td>Extraction Negative Control</td>
<td>PCR Negative Control</td>
<td>Standard A</td>
<td>Standard B</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

This configuration can be changed by selecting the desired well and changing its content from the Sample Type drop-down menu. For a sample, select Unknown.

7. Set up the name of each well by clicking over it and writing down the name in the appropriate field in the right side of the screen.

8. Click on the Save Protocol button to save the file to the computer.

9. Go to the Run section to see an overview of the assay setup.

10. If everything is correct, click on the Start button to run the run, which will take between 90 and 210 minutes depending on the virus that is aimed to be detected.

Adenovirus: 90 minutes
Hepatitis E: 210 minutes
Analysis of the Results

Once the run is finished, click on the Export Data button to automatically export the results to a Microsoft Excel spreadsheet.