Techne® qPCR test

Western equine encephalomyelitis virus
Structural polyprotein gene

150 tests

For general laboratory and research use only
Western equine encephalitis virus (WEEV) a member of the family Togaviridae, genus Alphavirus. Closely related to eastern and Venezuelan equine encephalitis viruses. These alphaviruses are spherical and have a diameter of 60-65 nm. The outer layer consists of a glycoprotein shell with protruding glycoprotein spikes, beneath which lies the lipid bilayer. The nucleocapsid core contains the single-stranded ribonucleic acid (RNA). The completed nucleotide sequence for WEE revealed an 11,508-nucleotide organism with an 84% concordance of protein similarity with EEEV. Our kit targets the virus’ structural polyprotein region.

These viruses are spread through the bite of an infected vector mosquito Culex tarsalis. Other mosquitoes and, occasionally, small, wild mammals also have been known to spread the virus. No cases of bird transmission of the disease have been reported, making mosquitoes the primary vector and birds simply reservoirs. Epidemic outbreaks in the equine or pheasant population often precede human epidemics of WEEV.

Symptoms range from mild flu-like illness to frank encephalitis, coma and death. cause severe inflammation of the brain (encephalitis) in horses and humans. These viruses primarily cause disease in equine species (e.g., horses, mules, donkeys, zebras), but a number of other animals such as pigs, llamas, bats, reptiles, amphibians, and rodents can also be infected. Birds are reservoirs for the virus, often being infected without signs of disease. Some birds (e.g., pheasants, emus, whooping cranes, partridges) can have illness or death once infected with WEEV.

Viral encephalitis viruses affect the nervous system, so affected animals will have fever, depression and changes in behaviour. Signs of infection may also include impaired vision, muscle twitches, circling or head pressing behaviours, the inability to swallow, paralysis and convulsions. Horses infected with EEE often do not survive. Survival rates of horses infected with WEEV is 70-80%.

People can be infected from the bite of an mosquito carrying the virus. Disease will vary depending on the specific virus involved. Signs include the sudden onset of fever, chills, body and joint aches. Infection can develop into severe encephalitis, resulting in headache, disorientation, tremors, seizures and paralysis. Permanent brain damage, coma and Vaccines for WEEV are available for horses. Measures to control mosquito populations and minimize mosquito exposure will decrease chances of infection.
Specificity

The Techne® qPCR Kit for Western equine encephalomyelitis virus (WEEV) genomes is designed for the in vitro quantification of WEEV genomes. The kit is designed to have the broadest detection profile possible whilst remaining specific to the WEEV genome. The primers and probe sequences in this kit have 100% homology with a broad range of WEEV sequences based on a comprehensive bioinformatics analysis.

If you require further information, or have a specific question about the detection profile of this kit then please send an e.mail to technehelp@bibby-scientific.com and our bioinformatics team will answer your question.
Kit Contents

• WEEV specific primer/probe mix (150 reactions BROWN)
  FAM labelled

• WEEV positive control template (for Standard curve RED)

• Internal extraction control primer/probe mix (150 reactions BROWN)
  VIC labelled as standard

• Internal extraction control RNA (150 reactions BLUE)

• Endogenous control primer/probe mix (150 reactions BROWN)
  FAM labelled

• WEEV/Internal extraction control/endogenous control RT primer mix (150 reactions GREEN)
  Required for two step protocol only

• RNAse/DNase free water (WHITE)
  for resuspension of primer/probe mixes and internal extraction control RNA

• Template preparation buffer (YELLOW)
  for resuspension of positive control template and standard curve preparation

Reagents and equipment to be supplied by the user

Real-Time PCR Instrument

RNA extraction kit
  This kit is redesigned to work well with all processes that yield high quality RNA
  with minimal PCR inhibitors.

Lyophilised OneStep 2x qRT-PCR MasterMix
  Contains complete one step qRT-PCR MasterMix

Pipettors and Tips

Vortex and centrifuge

1.5 ml tubes
Kit storage and stability
This kit is stable at room temperature but should be stored at -20ºC on arrival. Techne does not recommend using the kit after the expiry date stated on the pack. Once the lyophilized components have been re-suspended, unnecessary repeated freeze/thawing should be avoided. The kit is stable for six months from the date of resuspension under these circumstances.

If a standard curve dilution series is prepared this can be stored frozen for an extended period. If you see any degradation in this serial dilution a fresh standard curve can be prepared from the positive control.

Suitable sample material
All kinds of sample material suited for PCR amplification can be used. Please ensure the samples are suitable in terms of purity, concentration, and RNA/DNA integrity (An internal PCR control is supplied to test for non specific PCR inhibitors). Always run at least one negative control with the samples. To prepare a negative-control, replace the template RNA sample with RNase/DNase free water.

Dynamic range of test
Under optimal PCR conditions Techne WEEV detection kits have very high priming efficiencies of >95% and can detect less than 100 copies of target template.

Notices and disclaimers
This product is developed, designed and sold for research purposes only. It is not intended for human diagnostic or drug purposes or to be administered to humans unless clearly expressed for that purpose by the Food and Drug Administration in the USA or the appropriate regulatory authorities in the country of use. During the warranty period Techne® Prime Pro detection kits detection kits allow precise and reproducible data recovery combined with excellent sensitivity. For data obtained by violation to the general GLP guidelines and the manufacturer’s recommendations the right to claim under guarantee is expired. PCR is a proprietary technology covered by several US and foreign patents. These patents are owned by Roche Molecular Systems Inc. and have been sub-licensed by PE Corporation in certain fields. Depending on your specific application you may need a license from Roche or PE to practice PCR. Additional information on purchasing licenses to practice the PCR process may be obtained by contacting the Director of Licensing at Roche Molecular Systems, 1145 Atlantic Avenue, Alameda, CA 94501 or Applied BioSystems business group of the Applera Corporation, 850 Lincoln Centre Drive, Foster City, CA 94404. In addition, the 5' nuclease assay and other homogeneous amplification methods used in connection with the PCR process may be covered by U.S. Patents 5,210,015 and 5,487,972, owned by Roche Molecular Systems, Inc, and by U.S. Patent 5,538,848, owned by The Perkin-Elmer Corporation.

Trademarks
Techne™ is a trademark of Bibby Scientific Ltd.
The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG. BI, ABI PRISM® GeneAmp® and MicroAmp® are registered trademarks of the Applera Genomics (Applied Biosystems Corporation). BIOMEX® is a registered trademark of Beckman Instruments, Inc.; iCycler™ is a registered trademark of Bio-Rad Laboratories, Rotor-Gene is a trademark of Corbett Research. LightCycler™ is a registered trademark of the Idaho Technology Inc. GeneAmp®, TaqMan® and AmpliTaqGold® are registered trademarks of Roche Molecular Systems, Inc. The purchase of the Techne® Prime Pro reagents™ reagents cannot be construed as an authorization or implicit license to practice PCR under any patents held by Hoffmann-LaRoche Inc.
Principles of the test

Real-time PCR
A WEEV specific primer and probe mix is provided and this can be detected through the FAM channel.

The primer and probe mix provided exploits the so-called TaqMan® principle. During PCR amplification, forward and reverse primers hybridize to the WEEV cDNA. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5'-dye and a 3'-quencher. During PCR amplification, the probe is cleaved and the reporter dye and quencher are separated. The resulting increase in fluorescence can be detected on a range of real-time PCR platforms.

Positive control
For copy number determination and as a positive control for the PCR set up, the kit contains a positive control template. This can be used to generate a standard curve of WEEV copy number / CT value. Alternatively the positive control can be used at a single dilution where full quantitative analysis of the samples is not required. Each time the kit is used, at least one positive control reaction must be included in the run. A positive result indicates that the primers and probes for detecting the target WEEV gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. Care should be taken to ensure that the positive control does not contaminate any other kit component which would lead to false-positive results. This can be achieved by handling this component in a Post PCR environment. Care should also be taken to avoid cross-contamination of other samples when adding the positive control to the run. This can be avoided by sealing all other samples and negative controls before pipetting the positive control into the positive control well.

Negative control
To validate any positive findings a negative control reaction should be included every time the kit is used. For this reaction the RNAse/DNAse free water should be used instead of template. A negative result indicates that the reagents have not become contaminated while setting up the run. If a positive result is obtained the results should be ignored and the test samples repeated. Possible sources of contamination should first be explored and removed.

Internal RNA extraction control
When performing RNA extraction, it is often advantageous to have an exogenous source of RNA template that is spiked into the lysis buffer. This control RNA is then co-purified with the sample RNA and can be detected as a positive control for the extraction process. Successful co-purification and real-time PCR for the control RNA also indicates that PCR inhibitors are not present at a high concentration.

A separate RT primer mix and a real-time PCR primer/probe mix are supplied with this kit to detect the exogenous RNA using real-time PCR. The PCR primers are present at PCR limiting concentrations which allows multiplexing with the target sequence primers. Amplification of the control cDNA does not interfere with detection of the WEEV target cDNA even when present at low copy number. The Internal control is detected through the VIC channel and gives a CT value of 28+/-3 depending on the level of sample dilution.

Endogenous control
To confirm extraction of a valid biological template, a primer and probe mix is included to detect an endogenous gene. Detection of the endogenous control is through the FAM channel and it is NOT therefore possible to perform a multiplex with the WEEV primers. A poor endogenous control signal may indicate that the sample did not contain sufficient biological material.

Carry-over prevention using UNG (unsuitable for onestep procedure and optional for two step procedure)
Carry over contamination between PCR reactions can be prevented by including uracil-N-
glycosylase (UNG) in the reaction mix. Some commercial mastermix preparations contain UNG or alternatively it can be added as a separate component. UNG can only prevent carry over from PCR reactions that include deoxyuridine triphosphate (dUTP) in the original PCR reaction. Techne recommend the application of 0.2U UNG per assay with a 15 minute incubation step at 37°C prior to amplification. The heat-labile UNG is then inactivated during the Taq polymerase activation step.
Reconstitution Protocol

To minimize the risk of contamination with foreign DNA, we recommend that all pipetting be performed in a PCR clean environment. Ideally this would be a designated PCR lab or PCR cabinet. Filter tips are recommended for all pipetting steps.

1. Pulse-spin each tube in a centrifuge before opening. This will ensure lyophilised primer and probe mix is in the base of the tube and is not split upon opening the tube.

2. Reconstitute the kit components in the RNase/DNase free water supplied, according to the table below:

   To ensure complete resuspension, vortex each tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in water</th>
<th>Volume</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-PCR pack</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WEEV primer/probe mix (BROWN)</td>
<td>165 µl</td>
<td>165 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>165 µl</td>
<td>165 µl</td>
</tr>
<tr>
<td>RT primer mix (GREEN)</td>
<td>165 µl</td>
<td>165 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>165 µl</td>
<td>165 µl</td>
</tr>
<tr>
<td>Pre-PCR heat-sealed foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal extraction control RNA (BLUE)</td>
<td>600 µl</td>
<td></td>
</tr>
</tbody>
</table>

3. Reconstitute the positive control template in the template preparation buffer supplied, according to the table below:

   To ensure complete resuspension, vortex this tube thoroughly.

<table>
<thead>
<tr>
<th>Component - resuspend in template preparation buffer</th>
<th>Volume</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Post-PCR heat-sealed foil</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive Control Template (RED) *</td>
<td>500 µl</td>
<td></td>
</tr>
</tbody>
</table>

* This component contains high copy number template and is a VERY significant contamination risk. It must be opened and handled in a separate laboratory environment, away from the other components.

RNA extraction

The internal extraction control RNA can be added either to the RNA lysis/extraction buffer or to the RNA sample once it has been resuspended in lysis buffer.

DO NOT add the internal extraction control RNA directly to the unprocessed biological sample as this will lead to degradation and a loss in signal.

1. Add 4µl of the Internal extraction control RNA (BLUE) to each sample in RNA lysis/extraction buffer per sample.

2. Complete RNA extraction according to the manufacturers protocols.
One Step RT-PCR detection protocol

A one step approach combining the reverse transcription and amplification in a single closed tube is the preferred method.

For optimum performance and sensitivity.
All pipetting steps and experimental plate set up should be performed on ice. After the plate is poured proceed immediately to the One Step amplification protocol. Prolonged incubation of reaction mixes at room temperature can lead to PCR artifacts that reduce the sensitivity of detection.

1. For each RNA sample prepare a reaction mix according to the table below:
   Include sufficient reactions for positive and negative controls.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OneStep 2x qRT-PCR MasterMix</td>
<td>10 µl</td>
</tr>
<tr>
<td>WEEV primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>Internal extraction control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNAse/DNAse free water (WHITE)</td>
<td>3 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

2. For each RNA sample prepare an endogenous control reaction according to the table below (optional):
This control reaction will provide crucial information regarding the quality of the biological sample.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OneStep 2x qRT-PCR MasterMix</td>
<td>10 µl</td>
</tr>
<tr>
<td>Endogenous control primer/probe mix (BROWN)</td>
<td>1 µl</td>
</tr>
<tr>
<td>RNAse/DNAse free water (WHITE)</td>
<td>4 µl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td>15 µl</td>
</tr>
</tbody>
</table>

3. Pipette 15µl of these mixes into each well according to your real-time PCR experimental plate set up.

4. Pipette 5µl of RNA template into each well, according to your experimental plate set up.
   For negative control wells use 5µl of RNAse/DNAse free water. The final volume in each well is 20µl.
5. If a standard curve is included for quantitative analysis prepare a reaction mix according to the table below:

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>OneStep 2x qRT-PCR MasterMix</td>
<td>10 μl</td>
</tr>
<tr>
<td>WEEV primer/probe mix (BROWN)</td>
<td>1 μl</td>
</tr>
<tr>
<td>RNAse/DNase free water (WHITE)</td>
<td>4 μl</td>
</tr>
<tr>
<td><strong>Final Volume</strong></td>
<td>15 μl</td>
</tr>
</tbody>
</table>

6. Preparation of standard curve dilution series.

   1) Pipette 90μl of template preparation buffer into 5 tubes and label 2-6
   2) Pipette 10μl of Positive Control Template (RED) into tube 2
   3) Vortex thoroughly
   4) Change pipette tip and pipette 10 μl from tube 2 into tube 3
   5) Vortex thoroughly

   Repeat steps 4 and 5 to complete the dilution series

7. Pipette 5μl of standard template into each well for the standard curve according to your plate set-up
   The final volume in each well is 20μl.
### One Step Amplification Protocol
Amplification conditions using OneStep 2x RT-qPCR MasterMix.

<table>
<thead>
<tr>
<th>Step</th>
<th>Time</th>
<th>Temp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reverse Transcription</td>
<td>10 mins</td>
<td>42 °C</td>
</tr>
<tr>
<td>Enzyme activation</td>
<td>2 mins</td>
<td>95 °C</td>
</tr>
<tr>
<td>Denaturation</td>
<td>10 secs</td>
<td>95 °C</td>
</tr>
<tr>
<td>**DATA COLLECTION ***</td>
<td>60 secs</td>
<td>60 °C</td>
</tr>
</tbody>
</table>

* Fluorogenic data should be collected during this step through the FAM and VIC channels.
## Interpretation of Results

<table>
<thead>
<tr>
<th>Target</th>
<th>Internal control</th>
<th>Negative control</th>
<th>Positive control</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ive</td>
<td>+ive</td>
<td>-ive</td>
<td>+ive</td>
<td>+ive</td>
</tr>
<tr>
<td>+ive</td>
<td>-ive</td>
<td>-ive</td>
<td>+ive</td>
<td>+ive</td>
</tr>
<tr>
<td>-ive</td>
<td>+ive</td>
<td>-ive</td>
<td>+ive</td>
<td>-ive</td>
</tr>
<tr>
<td>-ive</td>
<td>-ive</td>
<td>-ive</td>
<td>-ive</td>
<td>Experiment fail</td>
</tr>
<tr>
<td>+ive</td>
<td>+ive</td>
<td>+ive</td>
<td>+ive</td>
<td>Experiment fail</td>
</tr>
</tbody>
</table>

### Internal PCR control

The CT value obtained with the internal control will vary significantly depending on the extraction efficiency, the quantity of RNA added to the RT and PCR reaction and the individual machine settings. CT values of $28\pm3$ are within the normal range. When amplifying a WEEV sample with a high genome copy number, the internal extraction control may not produce an amplification plot. This does not invalidate the test and should be interpreted as a positive experimental result.

### Endogenous control

The signal obtained from the endogenous control primer and probe set will vary according to the amount of biological material present in a given sample. An early signal indicates the presence of a good yield of biological material. A late signal suggests that little biological material is present in the sample.