

## Hop Stunt Viroid - Real Time

50 / 100 /150 tests (Ready to use kit)

AgroqPCR-realtime™ HSVd Real Time Kit is a screening assay for the detection of Hop Stunt Viroid.

### Principles of the test:

The Hop Stunt Viroid specific primer and probe mix are provided and this can be detected through the yellow channel.

During PCR amplification, forward and reverse primers hybridize to the Hop Stunt Viroid. A fluorogenic probe is included in the same reaction mixture which consists of a DNA probe labeled with a 5-dye Chods ZX™ and 3-quencher kurü Zy™. Internal control consists of a DNA probe labeled with a 5-dye kellú ZZ™ and a 3-quencher kurü Zy™.

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 realtime PCR platforms.

### Principle and use:

This amplification kit has been manufactured by Bioingentech Ltd. Chile to detect Hop Stunt Viroid in real time PCR. This is a possibility absolute quantification or qualitative assay. Real time PCR is based on fluorogenic dyes. Up to 36 Ct should be taken positive. Value between 36-40 Ct should be taken as marginal positive.

This kit needs RNA which can be isolated from blood, serum, faeces, respiratory fluid, digestive system, tissue and others. See (PU-A003, PU-A004 & PU-A006) included in advanced formats.

### Kit Components:

Pulse-spin each tube in a centrifuge before opening. Completely thaw the components of the kit prior to use. Homogenize the solutions for 5 seconds prior to pipetting.

| Reactions                           | 50 test  | 100 test | 150 test |
|-------------------------------------|----------|----------|----------|
| cDNA Synthesis Premixture           | (1 vial) | (1 vial) | (1 vial) |
| AgroqPCR-realtime™ HSVd             | (1 vial) | (1 vial) | (1 vial) |
| Lig™ RNase Inhibitor Solution       | (1 vial) | (1 vial) | (1 vial) |
| Bio™ Transcriptase Solution         | (1 vial) | (1 vial) | (1 vial) |
| PCR Grade water                     | (1 vial) | (1 vial) | (1 vial) |
| HSVd Positive Control               | (1 vial) | (1 vial) | (1 vial) |
| HSVd Negative Control               | (1 vial) | (1 vial) | (1 vial) |
| AgroqPCR-realtime™ Internal Control | (1 vial) | (1 vial) | (1 vial) |
| Mineral Oil                         | (1 vial) | (1 vial) | (1 vial) |

### Instrument Compatibility in:

|                     |                             |
|---------------------|-----------------------------|
| * ABI 7300          | * LightCycler 2.0           |
| * ABI 7500FAST      | * LightCycler 480           |
| * ABI 7900          | * Mastercycler® ep realplex |
| * AB Step One       | * Mx3000P QPCR System       |
| * AB Step One Plus  | * Mx3005P QPCR System       |
| * Agilent Mx3005P   | * RotorGene 3000            |
| * CFX96 & CFX384    | * RotorGene 6000            |
| * ExiCycler™ 96     | * RotorGeneQ                |
| * iQ5 & MyiQ Cycler | * SLAN® Real-Time PCR       |
| * Illumina Eco      | * Smartcycles II            |
| * LightCycler Nano  | * Applied 7300 and 7500     |

### Procedure:

Please read through the entire procedure before starting.

#### Step 1

#### Preparation of First Strand cDNA Synthesis:

1) Prepare the reaction mixture for sample by combining the reagents as shown in the table 1.

Table 1. Components for cDNA synthesis

| Kit components               | samples |
|------------------------------|---------|
| cDNA Synthesis Premixture    | 4.5 µL  |
| PCR Grade water              | 4µL     |
| RNA isolated from the sample | 2µL     |



Sample: 2µl RNA (see PU-A004, PU-A005 or PU-A006)  
4µl PCR Grade water  
4.5µl cDNA Synthesis Premixture

#### Notes:

• The mineral oil is necessary, even when using a thermal cycler that employs a top heating method.



Sample: A Mineral Oil Drop  
2µl RNA (see PU-A004, PU-A005 or PU-A006)  
4µl PCR Grade water  
4.5µl cDNA Synthesis Premixture

**Total: 10,5 µL**

• The reactive necessary to cDNA Synthesis should be 10.5µl.


## Step 2

2) Place the tubes in a thermal cycler and perform Reverse Transcription according to the program outlined in Table 2.

Table 2. Reverse Transcription

|           |         |                      | Temp  | Time    |
|-----------|---------|----------------------|-------|---------|
| Program 1 | 1 cycle | Initial denaturation | 65 °C | 10 min. |
|           | 1 cycle | Stop                 | 4 °C  | 5 min.  |

|            |                               |
|------------|-------------------------------|
| Add 1.0 µl | Lig™ RNase Inhibitor Solution |
| Add 1.0 µl | Bio™ Transcriptase Solution   |


Sample:  2µL RNA (se PU-A004, PU-A005 or PU-A006)  
4µL PCR Grade Water  
4,5 µL cDNA Synthesis Premixture  
1µL LigTm RNase Inhibitor Solution  
1µL BioTm Transcriptase Solution

**Total: 13,5 µL**

Then use this program

|           |         |              | Temp  | Time    |
|-----------|---------|--------------|-------|---------|
| Program 2 | 1 cycle | Annealing    | 25 °C | 10 min. |
|           | 1 cycle | Extension    | 37°C  | 60 min. |
|           | 1 cycle | Denaturation | 70 °C | 10 min. |

After this program achieved: cDNA from the sample.


Sample:  → cDNA from the sample


## Step 3


Table 3. Reaction components for Real Time PCR


|                                      | Samples | Positive control | Negative control | Internal control |
|--------------------------------------|---------|------------------|------------------|------------------|
| AgroqPCR-realttime™ HSVd             | 5.5 µL  | 5.5 µL           | 5.5 µL           |                  |
| AgroqPCR-realttime™ Internal Control |         |                  |                  | 5.5 µL           |
| PCR Grade water                      | 6µL     | 6µL              | 6µL              | 6µL              |
| cDNA from the sample                 | 2µL     |                  |                  | 2µL              |
| HSVd Positive Control                |         | 2µL              |                  |                  |
| HSVd Negative Control                |         |                  | 2µL              |                  |
| Total Volumen                        | 13.5µL  | 13.5µL           | 13.5µL           | 13.5µL           |

## Visual explanation:

Sample:  2µL cDNA from the sample  
6µL PCR Grade water  
5.5µL AgroqPCR-realttime™ HSVd  
**Total: 13,5 µL**

Positive Control:  2µL HSVd Positive Control  
6µL PCR Grade water  
5.5µL AgroqPCR-realttime™ HSVd  
**Total: 13,5 µL**

Negative Control:  2µL HSVd Negative Control  
6µL PCR Grade water  
5.5µL AgroqPCR-realttime™ HSVd  
**Total: 13,5 µL**

Internal Control:  2µL cDNA from the sample  
6µL PCR Grade water  
5.5µL AgroqPCR-realttime™ Internal Control  
**Total: 13,5 µL**

Observation: homogenize solution 10 seconds.

3) Place the tubes in a Instrument and perform amplification according to the program outlined in Table 4.

Table 4. Real Time cycling parameters

| Real Time PCR cycle | Temp.                | Time            |
|---------------------|----------------------|-----------------|
| x 1 cycles          | Initial Denaturation | 94°C 2 min.     |
| x 45 cycles         | Denaturation         | 95°C 15 seconds |
|                     | Annealing            | 60°C 60 seconds |

## Interpretation of the test

### 1) Qualitative analysis:

Ct (Threshold cycle) value of each sample can be read as follows.

Ct value result

|               |               |
|---------------|---------------|
| > 40 negative | ≤ 40 positive |
|---------------|---------------|

## 2) Quantitative analysis:

Table 3. Preparation of standard curve dilution series. HSVd positive control:

| Average Positive Control Concentration |                     |
|--|---------------------|
| <b>HSVd</b>                            | See quality control |

| Standar curve | Preparation series a fresh dilution                          | Concentration       | Copy Number         |
|---------------|--|---------------------|---------------------|
| Tube N°1:     | 2uL HSVd Positive Control (0,3 ng/μl) + 18uL PCR Grade Water | See quality control | See quality control |
| Tube N°2:     | 2uL Tube N°1 + 18uL PCR Grade Water                          | See quality control | See quality control |
| Tube N°3:     | 2uL Tube N°2 + 18uL PCR Grade Water                          | See quality control | See quality control |
| Tube N°4:     | 2uL Tube N°3 + 18uL PCR Grade Water                          | See quality control | See quality control |
| Tube N°5:     | 2uL Tube N°4 + 18uL PCR Grade Water                          | See quality control | See quality control |
| Tube N°6:     | 2uL Tube N°5 + 18uL PCR Grade Water                          | See quality control | See quality control |
| Tube N°7:     | 2uL Tube N°6 + 18uL PCR Grade Water                          | See quality control | See quality control |

Homogenize tube.

**\* We will send a Quality Control report for each purchase**  
**\*\* If you want to obtain 13 DNA copies you must include a new dilution tube (Tube N° 8)**

Table 4. Reaction components for Real Time PCR standar curve.

|                 | Tube A | Tube B | Tube C | Tube D | Tube E | Tube F | Tube G |
|-----------------|--------|--------|--------|--------|--------|--------|--------|
| AgroqPCR HSVd   | 5.5 μl | 5.5 μl | 5.5 μl | 5.5 μl | 5.5 μl | 5.5 μl | 5.5 μl |
| PCR Grade Water | 6.0 μl | 6.0 μl | 6.0 μl | 6.0 μl | 6.0 μl | 6.0 μl | 6.0 μl |
| Tube N°1        | 2.0 μl |        |        |        |        |        |        |
| Tube N°2        |        | 2.0 μl |        |        |        |        |        |
| Tube N°3        |        |        | 2.0 μl |        |        |        |        |
| Tube N°4        |        |        |        | 2.0 μl |        |        |        |
| Tube N°5        |        |        |        |        | 2.0 μl |        |        |
| Tube N°6        |        |        |        |        |        | 2.0 μl |        |
| Tube N°7        |        |        |        |        |        |        | 2.0 μl |

2.1.- Assess the Ct value when amplification curve of Standard tube 1, 2, 3, 4, 5, 6 passes the threshold line. However four tubes is sufficient for standard curve. (tube1-tube4).

2.2.- Calculate quantitative value to compare with Ct value of unknown samples and curve of Standard tube 1, 2, 3, 4, 5, 6.

### 3) Test validation:

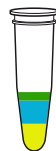
3.1.- Each Ct value standard should be as follows.  
 Standard 1 > Standard 2 > Standard 3 > Standard 4 > Standard 5 > Standard 6.

3.2.- R-value of standard curve should be 0.900~0.999.

3.3.- The standard result should be all negative.

### Visual explanation:

#### Tube A



2.0μl Tube N°1  
 6.0μl PCR Grade Water  
 5.5μl AgroqPCR-realtime™ HSVd

**Total: 13,5 μL**

#### Tube B



2.0μl Tube N°2  
 6.0μl PCR Grade Water  
 5.5μl AgroqPCR-realtime™ HSVd

**Total: 13,5 μL**

#### Tube C



2.0μl Tube N°3  
 6.0μl PCR Grade Water  
 5.5μl AgroqPCR-realtime™ HSVd

**Total: 13,5 μL**

#### Tube D



2.0μl Tube N°4  
 6.0μl PCR Grade Water  
 5.5μl AgroqPCR-realtime™ HSVd

**Total: 13,5 μL**

## Tube E



2.0µl Tube N°5  
6.0µl PCR Grade Water  
5.5µl AgroqPCR-realtime™ HSVd

**Total: 13,5 µL**

## Tube F



2.0µl Tube N°6  
6.0µl PCR Grade Water  
5.5µl AgroqPCR-realtime™ HSVd

**Total: 13,5 µL**

## Tube G



2.0µl Tube N°6  
6.0µl PCR Grade Water  
5.5µl AgroqPCR-realtime™ HSVd

**Total: 13,5 µL**

## FAQ:

### 1.-Positive control:

The positive control assay uses a Chods Zx™ dye and should be detected through the Yellow channel of your real time PCR instrument.

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 Hop Stunt Viroid copy number / CT value. Alternatively the positive control can be used at a single dilution Hop Stunt Viroid on where full quantitative analysis of the sample 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 primer and probes for detecting the target Hop Stunt Viroid gene worked properly in that particular experimental scenario. If a negative result is obtained the test results are invalid and must be repeated. sealing all other samples and negative controls before pipetting the positive control into the positive control well tube.

### 2.-Internal Control:

The internal control assay uses a **kellú ZZ™** dye and should be detected through the **Green** channel of your real time PCR instrument. A positive result through the **Green** channel therefore indicates that PCR conditions are suitable for detection of the target pathogen gene. If a negative result is obtained through the **Green** channel the results should be ignored and the test repeated.

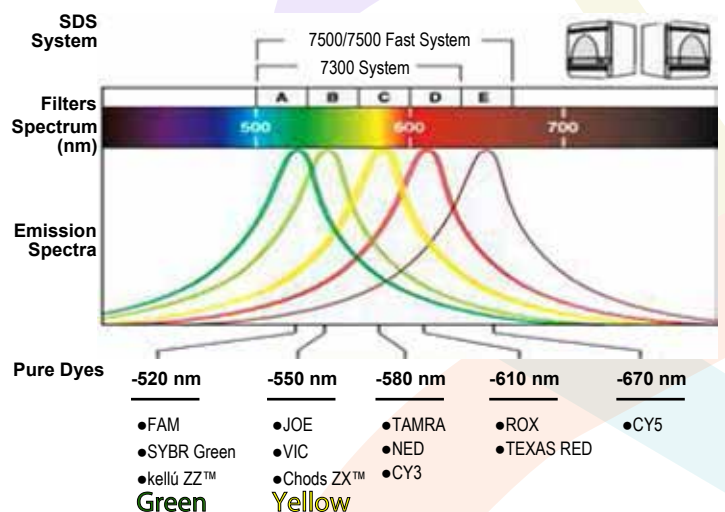
### 3.-Negative control:

To confirm absence of contamination a negative control reaction should be included every time the kit is used. In this instance the PCR grade water should be used in place of template. A negative result indicates that the reagents have not become contaminated. If a positive result (CT value less than 37) is obtained the results should be ignored and the test repeated.

You must use quencher and reporter dye to setup your software (see table 2) and run the following channel:

Table 7. Report Dye channel selection chart

| Channel | Source   | Detector | Dyes   |
|---------|----------|----------|--|
| Green   | 470 (Nm) | 520 (Nm) | FAM, Sybr green1, Fluorescein, Eva green, Alerxa flour 488, <b>kellú ZZ™</b> |
| Yellow  | 530 (Nm) | 550 (Nm) | Joe, Vic, Hex, Tet, Cal Fluorgold 540, YaKima Yellow, Chods ZX™              |
| Orange  | 585 (Nm) | 610 (Nm) | Rox, Cal Fluor Red 610, Cy3.5, Texas Red, Alexa Fluor 568                    |
| Red     | 625 (Nm) | 660 (Nm) | Cy5, Quasar 670, Lightcycler, Red 640, Alexa Fluor 633.                      |
| Crimson | 680 (Nm) | 710 (Nm) | Quasar 705, Lightcycler Red 705, Alexa Fluor 680                             |



Similarity of our fluorophores with HEX and FAM.

|            |     |
|------------|-----|
| ●kellú ZZ™ | FAM |
| ●Chods ZX™ | HEX |

## Temperature

All our reagents are made through protein engineering and are stable at room temperature, the label temperature is just a recommendation after the product is open.


|  |                      |                       |
|--|----------------------|-----------------------|
|  | Store temperature    | The label temperature |
|  | Shipping temperature | At room temperature   |

Table 8. Interpretation of Results

| Target | Internal Control | Negative Control | Positive Control | Interpretation  |
|--------|------------------|------------------|------------------|-----------------|
| +ive   | +ive             | -ive             | +ive             | +ive            |
| +ive   | -ive             | -ive             | +ive             | +ive            |
| +ive   | +ive             | +ive             | +ive             | *               |
| +ive   | -ive             | +ive             | +ive             | *               |
| -ive   | +ive             | -ive or +ive     | +ive             | -ive            |
| -ive   | -ive             | -ive or +ive     | -ive             | Experiment fail |
| -ive   | +ive             | -ive or +ive     | -ive             | Experiment fail |

Table 9. Products

| Products   | Code    |
|--|---------|
| Bioingentech - Genomic RNA Purification Kit 50 test  | PU-A004 |
| Bioingentech - Genomic RNA Purification Kit 100 test   | PU-A005 |
| Bioingentech - Genomic RNA Purification Kit 150 test   | PU-A006 |
| AgroqPCR-realtime™ HSVd<br>Hop Stunt Viroid Real Time<br>50 tests (Ready to use kit) / Cat. No: RTq-A545-50R   |         |
| AgroqPCR-realtime™ HSVd<br>Hop Stunt Viroid Real Time<br>100 tests (Ready to use kit) / Cat. No: RTq-A545-100R |         |
| AgroqPCR-realtime™ HSVd<br>Hop Stunt Viroid Real Time<br>150 tests (Ready to use kit) / Cat. No: RTq-A545-150R |         |