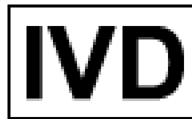
Geno-Sen's

WEST NILE VIRUS

Real Time PCR Kit

Quantitative/Qualitative



PACK INSERT

Genome Diagnostics Pvt. Ltd.

(An ISO 13485:2012, 9001:2008 Certified Company)

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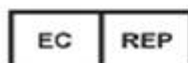

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WEST NILE VIRUS Geno-Sen's Real Time PCR Kit for use

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1. Contents of the Kit:

Color Code	Contents	REF 9111061 100 rxns	REF 9111062 50 rxns	REF 9111063 25 rxns
R1 Blue	WNV Super mix.	25 rxns x 4 Vials	25 rxns x 2 Vials	25 rxns x 1 Vials
R2 Yellow	Mg Sol RT.	1 Vial	1 Vial	1 Vial
WNV- S1 Red	*WEST NILE VIRUS Standard 1 1 X 10^5 copies/µl*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
WNV- S2 Red	*WEST NILE VIRUS Standard 2 1 X 10^4 copies/µl*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
WNV- S3 Red	*WEST NILE VIRUS Standard 3 1 X 10^3 copies/µl*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
WNV- S4 Red	*WEST NILE VIRUS Standard 4 1 X 10^2 copies/µl*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
WNV- S5 Red	*WEST NILE VIRUS Standard 5 1 X 10^1 copies/µl*	1 Vial of 300µl	1 Vial of 300µl	1 Vial of 300µl
W White	Molecular Grade Water.	1 Vials of 1 ml	1 Vial of 1 ml	1 Vial of 1 ml
IC-1 (R3) Green	IC-1 RG (R3)	1 Vial of 1 ml	1 Vial of 1 ml	1 Vial of 1 ml

R = Reagents
S = Quantitation Standards
W = Molecular Grade Water.

All Vials have Color Coder tops to distinguish between different reagents.

2. Storage of the Kit.

All the reagents of the kit should be stored at -20°C and is stable till expiry at this temperature. Repeated thawing and freezing (> 3x) should be avoided, as this may reduce the sensitivity of the assay. If the kit is to be used only occasionally, the reagents should be frozen in aliquots. Storage at +4°C is not recommended & should not exceed a period of 2 hours in any case.

3. WEST NILE VIRUS Information

Application

West Nile encephalitis is an infection of the brain that is caused by a virus known as the West Nile virus. This virus was first identified in Uganda in 1937. It is commonly found in Africa, West Asia, and the Middle East. West Nile virus also is called West Nile fever or West Nile encephalitis.

Signs and symptoms of the West Nile virus infection range from no symptoms at all to a rapidly fatal brain infection. In areas where the virus is common, people are more likely to show no symptoms of the infection or have only a mild, flu like illness rather than a severe brain infection. Even in an area of outbreak, the likelihood of a person developing illness after infection with West Nile virus is about 1 in every 140-300 people.

West Nile virus infection typically begins with the abrupt onset of fever, chills, muscle aches, headache, and overall feeling of illness. Headache is particularly common and may be severe. The person may have sensitivity to light with pain behind the eyes. Most people fully recover. In others, particularly the elderly, the disease can progress to cause encephalitis or meningitis. In the 59 people hospitalized during the initial New York outbreak, signs and symptoms included fever (90%), muscle weakness (54%), headache (46%), altered mental status (44%), rash (22%), stiff neck (19%), joint aches (17%), sensitivity to light (15%), and body aches (14%).

The *Geno-Sen's* WEST NILE VIRUS Quantification assay is developed for laboratory scale or high-throughput quantitative transcript analysis by real time quantitative fluorescence PCR.

Geno Sen's standardized ready-to-use Control and Reaction mix allow fast processing of the samples.

Samples which can be used for Extraction: Serum, plasma, whole blood, CSF etc.

4. Precautions for PCR

The following aspects should always be taken care of:

- Store positive material (Specimens, Standards or amplicons) separately from all other reagents and add it to the reaction mix in a separate facility.
- Thaw all components thoroughly at room temperature before starting the assay.
- When thawed, mix the components and centrifuge briefly.
- Work quickly on ice or in the Cooling Block.

- All the reagents including the NTC (except for standards & specimens) should be mixed & dispensed in pre-mix area.
- All the standards & specimens should be mixed & dispensed in extraction area.
- Use pipette tips with filters only.
- Always use disposable powder-free gloves

5. Additionally Required Materials and Devices

- RNA isolation kit (see **8.a. RNA extraction**)
- 0.2 ml PCR tubes for use with 36-well rotor (Corbett Research, Cat.-Nr.: SE-1003F) alternatively 0.1 ml PCR tubes for use with 72-well rotor (Corbett Research, Cat.-Nr.: ST-1001)
- Micro Pipettes Variable Volume 2-20µl, 10-100µl, 100-1000µl,
- Sterile pipette tips with aerosol barrier 2-20µl, 10-100µl, 100-1000µl,
- Disposable powder-free gloves
- Vortex mixer
- Centrifuge Desktop with rotor for 1.7 ml reaction tubes
- *Rotor Gene™ 2000,3000* or *Rotor Gene™ 6000*, Corbett Research (The Real time PCR Instrument)

6. Principle of Real-Time PCR

The robust assay exploits the so-called Taqman principle. During PCR, forward and reverse primers hybridize to a specific sequence product. A TaqMan probe, which is contained in the same reaction mixture and which consists of an oligonucleotide labeled with a 5'-reporter dye and a downstream, 3'-quencher dye, hybridizes to a target sequence within the PCR product. A Taq polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. The reporter dye and quencher dye are separated upon cleavage, resulting in an increase in fluorescence for the reporter. Thus, the increase in fluorescence is directly proportional to the target amplification during PCR.

7. Description Of the Product.

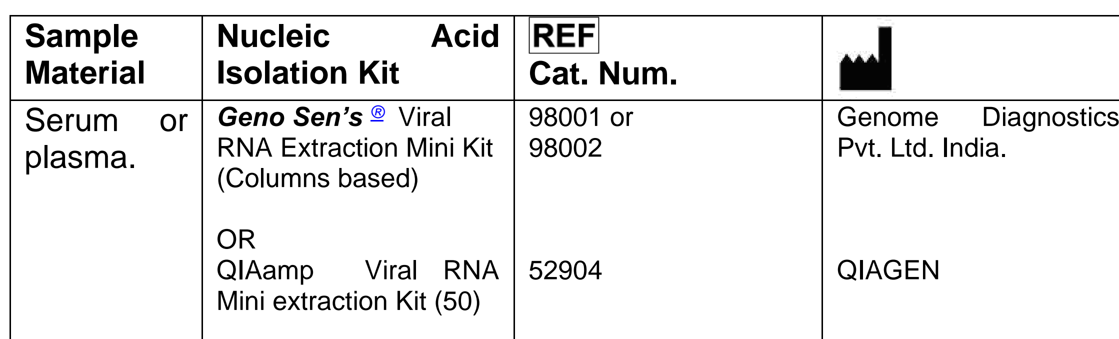
The ***Geno-Sen's West Nile Virus*** *PCR* Reagents constitute a ready to use system for detection and quantification of West Nile Virus using Polymerase chain reaction (PCR) in the Rotor Gene 2000/3000/6000 (Corbett Research). *The Specific Master* mix contains reagents and enzymes for the specific amplification of West Nile Virus and for the direct detection of the specific amplicon in fluorescence channel Cycling

A.FAM of the *Rotor Gene 2000/3000/6000 & the Reference gene on* Cycling A. Joe. External positive Standards (WNV S 1-5) are supplied which allow the determination of the gene load. For further information, please refer to section 8.c Quantitation.

8. Procedure

8.a RNA Extraction

RNA Extraction kits are available from various manufacturers. Sample volumes for the RNA Extraction procedure depend on the protocol used. Please carry out the RNA Extraction according to the manufacturer's instructions. The recommended Extraction kits are the following:



Sample Material	Nucleic Acid Isolation Kit	REF Cat. Num.	
Serum or plasma.	***Geno Sen's*** ® Viral RNA Extraction Mini Kit (Columns based) OR QIAamp Viral RNA Mini extraction Kit (50)	98001 or 98002 52904	Genome Diagnostics Pvt. Ltd. India. QIAGEN

The ***Geno Sen's***® West Nile Real Time PCR kits has been optimized with the above mentioned extraction kit. The ***Geno Sen's***® RNA Extraction Mini Kit provides a relatively higher yield than most of the commercial extraction kits available on the world market & hence is the preferred Kit for extraction of Viral RNA. However the customer can use their own extraction systems depending on how good the yield is. However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher RNA yield.

Always use an extraction kit with a higher RNA yield otherwise the low positives will not be detected.

However the customer can use their own extraction systems depending on how good the yield is. Always use an extraction kit with a higher RNA yield.

Blood collection tubes coated with anticoagulants may inhibit the PCR, However these inhibitors will be eliminated by the use of the isolation kits given above. It is recommended to avoid the usage of heparin blood.

When using Extraction protocols with ethanol-containing washing buffers, please carry out an additional centrifugation step before the elution to remove any remaining ethanol. This prevents possible inhibition of PCR.

The *West Nile Virus Rotor Gene PCR Reagents* should not be used with phenol based isolation methods.

8.b *Inhibition Control:*

Inhibition Control Gene allows the user to determine & control possible PCR inhibition. The Inhibition Control gene reagents are in built in the premix provided and need not be run separately. There is need to add internal control Gene in the reaction mix which has been provided as IC-1 RG (R3). To add the IC Gene 0.5 µl/rxn of R3 is added to the premix as shown in the Figure 4 & 5. The results can be visualized in the Joe channel.

8.c Quantitation

The quantitation standards provided in the kit *(WNV S 1-5)* are treated in the same way as extracted samples and the same volume is used i.e. (15µl) instead of the sample. To generate a standard curve in the *RotorGene™ 2000/3000/6000*, all 5 Standards should be used as defined in the menu window *Edit Samples* of the *RotorGene™* software. The same should also be defined as standards with the specified concentrations (see *RotorGene™* Manual). The standard curve generated as above can also be used for quantitation in subsequent runs, provided that at least one standard is used in the current run. For this purpose, the previously generated standard curve needs to be imported (see *Rotor Gene™ 2000/3000/6000 Manual*). However, this quantitation method may lead to deviations in the results due to variability between different PCR runs & due to varying Reaction efficiencies.

Attention: The standards are defined as Copies/µl. The following formula has to be applied to convert the values determined using the standard curve into Copies/ml of sample material:

$$\textbf{Result (Copies/ml)} = \frac{\textbf{Result (Copies/µl) x Elution Volume (µl)}}{\textbf{Sample Volume (ml)}}$$

Or else direct conversion can be done keeping in mind the starting volume of the sample & the final eluted Volume e.g.

In case ***Geno Sen's***® Viral RNA Extraction Mini Kit is being used where the starting volume is 150µl & the final Eluted Volume is 60µl then to obtain the direct values i.e.

copies/ml for the patient samples the following values for the standards can be fed directly into the operating software which is again based on the above formula and will yield direct conversion of per ml of patient sample data.

S1: 10^5 **copies**/µl = 40000000 **copies**/ml
S2: 10^4 **copies**/µl = 4000000 **copies**/ml
S3: 10^3 **copies**/µl = 400000 **copies**/ml
S4: 10^2 **copies**/µl = 40000 **copies**/ml
S5: 10^1 **copies**/µl = 4000 **copies**/ml

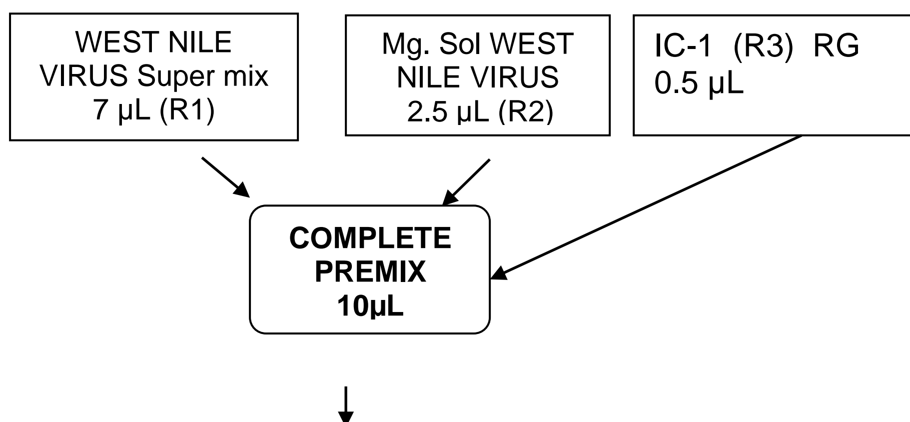
If the starting volume of the sample while using the Qiagen QIAamp Viral RNA Mini extraction kit is 140µl & the final Eluted Volume is 50µl then to obtain the direct values i.e. Copies/ml for the patient samples the following values for the standards can be fed directly into the operating software which is again based on the above formula and will yield direct conversion of per ml of patient sample data.

S1: 10^5 Copies/µl = 35750000 Copies/ml
S2: 10^4 Copies/µl = 3575000 Copies/ml
S3: 10^3 Copies/µl = 357500 Copies/ml
S4: 10^2 Copies/µl = 35750 Copies/ml
S5: 10^1 Copies/µl = 3575 Copies/ml

8.d Preparation for PCR & amplification.

First make sure that the Cooling Block (accessory of the *Rotor Gene*™, Corbett Research) is pre-cooled to +4°C in a Refrigerator or Deep Freezer. Place the desired number of PCR tubes into the Cooling Block. Make sure that the tubes for standards & at least one negative control (*Water, PCR grade*) are included per PCR run. To generate a standard curve, use all supplied Standards *(WNV S 1-5)* for each PCR run. Before each use, all reagents need to be thawed completely and mixed (by pipetting or by brief vortexing).

Please follow the pipeting scheme mentioned below for each sample depending upon the number of samples a mix can be prepared as follows. Each sample



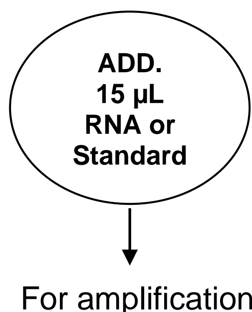


Fig. 4.

Depending upon the number of samples the following pipetting scheme can be followed. e.g. for 10 rxns.

WEST NILE VIRUS MASTER MIX	1 rxns.	10 rxns.
WEST NILE VIRUS Super Mix (R1)	7 µL	70 µL
WEST NILE VIRUS Mg Sol. (R2)	2.5 µL	25 µL
IC-1 (R3) RG	0.5 µL	5 µL
Total	10µL	100µL

Fig. 5.

Pipette 10 µl of the Master Mix into each labelled PCR tube. Then add 15 µl of the earlier extracted RNA to each sample tube and mix well by pipeting up and down. Correspondingly, 15 µl of the Standards (WEST NILE VIRUS *S1-5*) must be used as a positive control and 15 µl of water (*Water, PCR grade*) as a negative control. Close the PCR tubes and transfer the same into the rotor of the *RotorGene*™ instrument. The *RotorGene*™ software versions 5.0.53 and higher require a Locking Ring (accessory of the *RotorGene*™, Corbett Research) to be placed on top of the rotor to prevent accidental opening of the tubes during the run.

8.e. Programming of the instrument

The program for the detection of West Nile Virus can be divided into following steps:

A. Setting of general assay parameters & reaction volume
B. Thermal Profile & Calibration
C. Cycling profile/ cDNA Synthesis & Initial activation of the Hot Start enzyme
D. Cycling for Amplification of cDNA
E. Adjustment of the sensitivity of the fluorescence channels
F. Starting of the *Rotor Gene*™ run

Program the *RotorGene*™ 6000 for these 5 steps according to the parameters shown in Figures 22-39 below All specifications refer to the *RotorGene*™ 6000 software version 1.7 Please find further information on programming the *RotorGene*™ in the *RotorGene*™ 6000 *Operator's Manual*,. In the illustrations these settings are shown by arrows

g) Setting of general assay parameters & Reaction volume.

Please see to it that you are in advanced mode and then click Hydrolysis Probes.

On a double click the software will automatically go to the next function. If there is just a single click then click new after highlighting the Hydrolysis Probes.

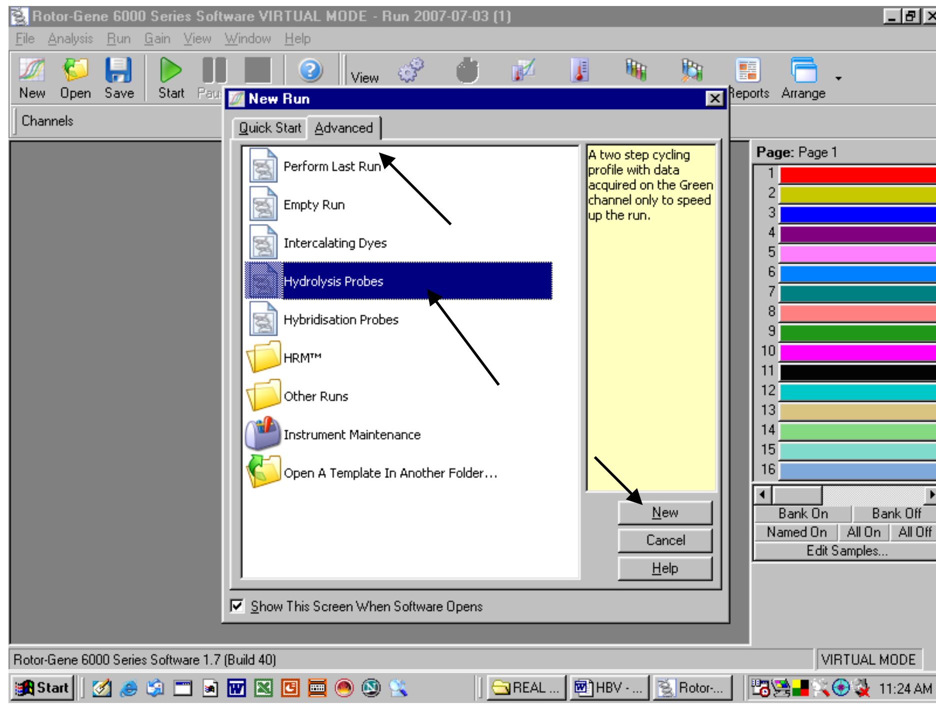


Fig. 22.

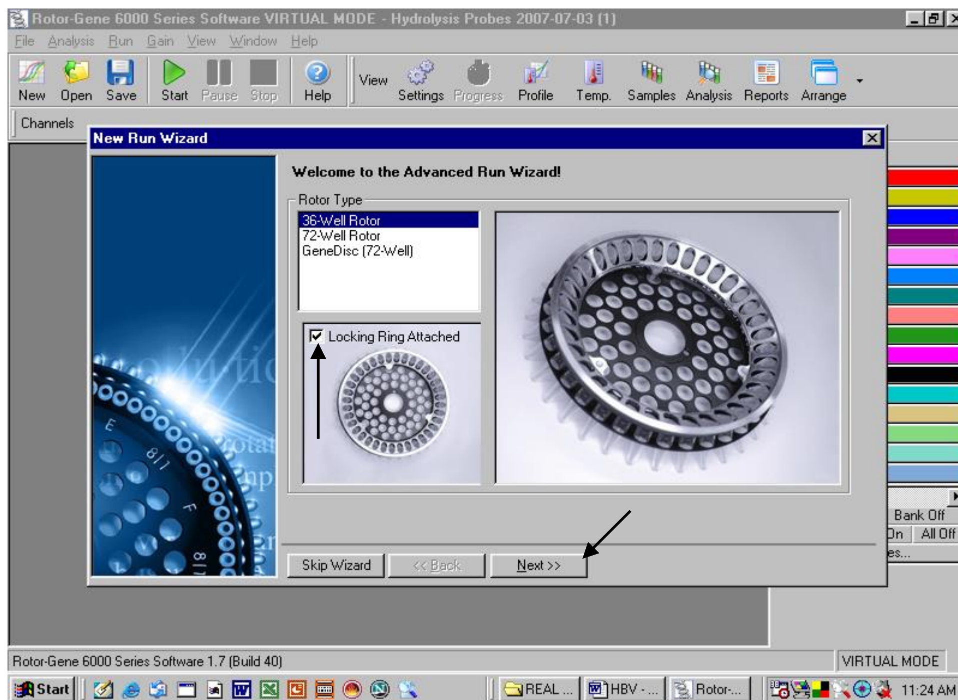


Fig. 23.

First, confirm by clicking in the box as shown in the figure above whether a locking ring has been attached. Then click next to proceed to the next step.

Confirmation of reaction Volume as follows.

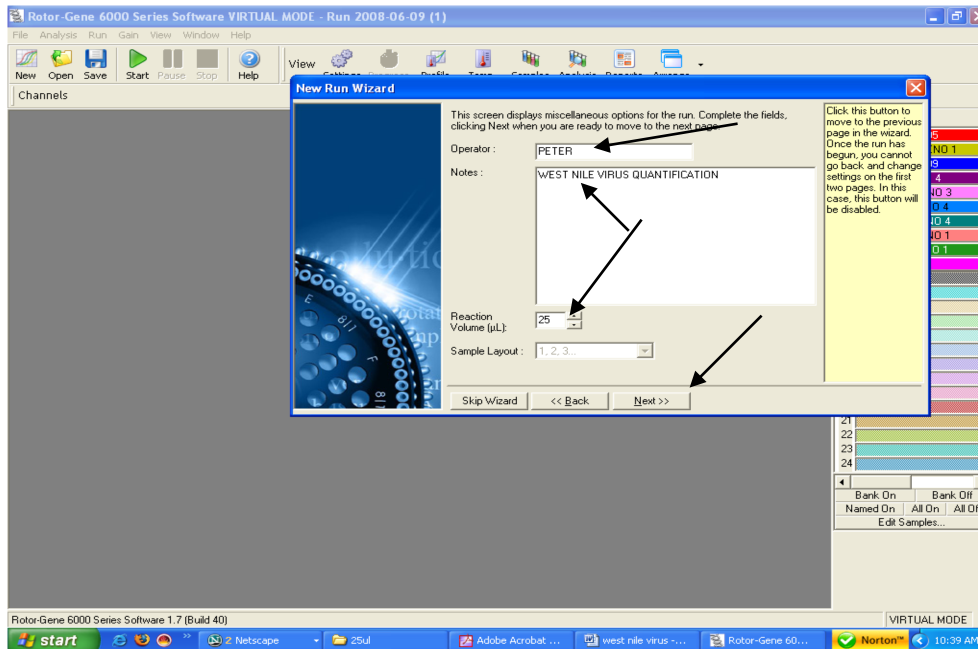


Fig. 24.

- Please click on the volume buttons to make sure that 25µl is reflected in the window as shown above.
- In case required, Operators Name can be Fed into the system.
- Any Notes which need to be either printed into the Report can be typed out in the Box of Notes.
- Then click next and a new window will open as shown below.

h) THERMAL PROFILE & CALIBRATION:

Here the thermal profile for the assay will be defined.

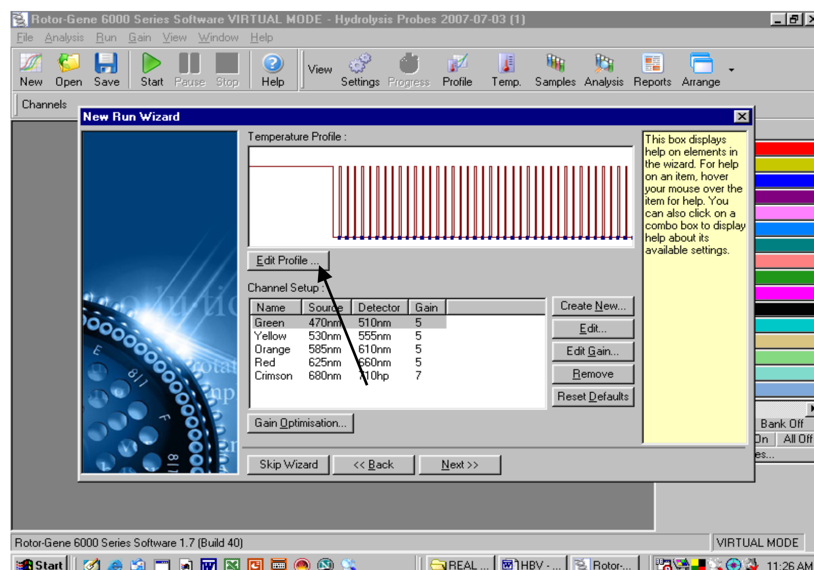


Fig. 25.

Programming the temperature profile is done by activating the button *Edit Profile* in the next *New RUN Wizard* menu window as shown above.

i) **CYCLING PROFILE: First hold** 50°C for 15 minutes i.e. cDNA synthesis step as below

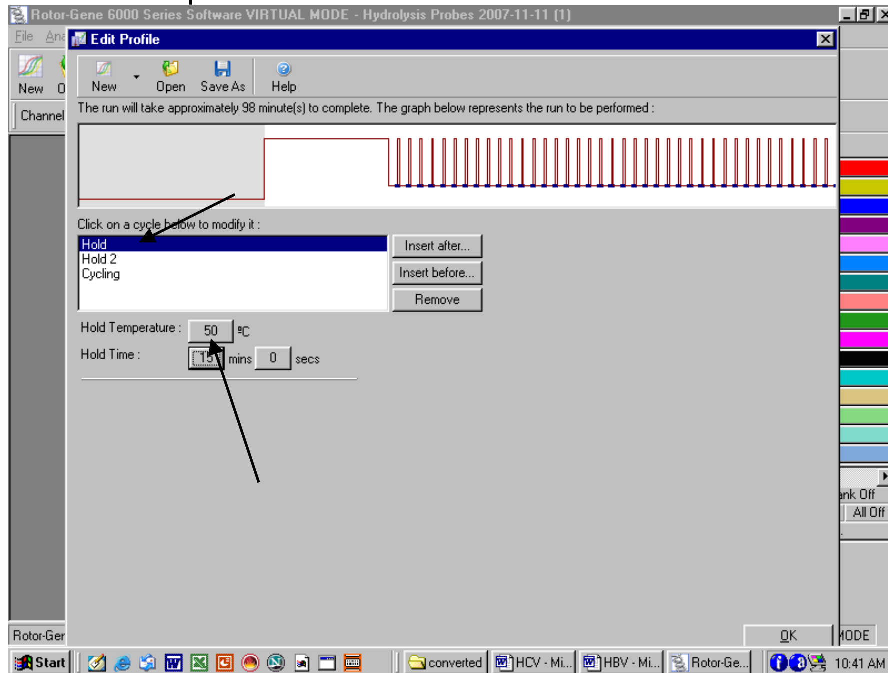


Fig. 26.

Second hold 95°C for 10 minutes as below

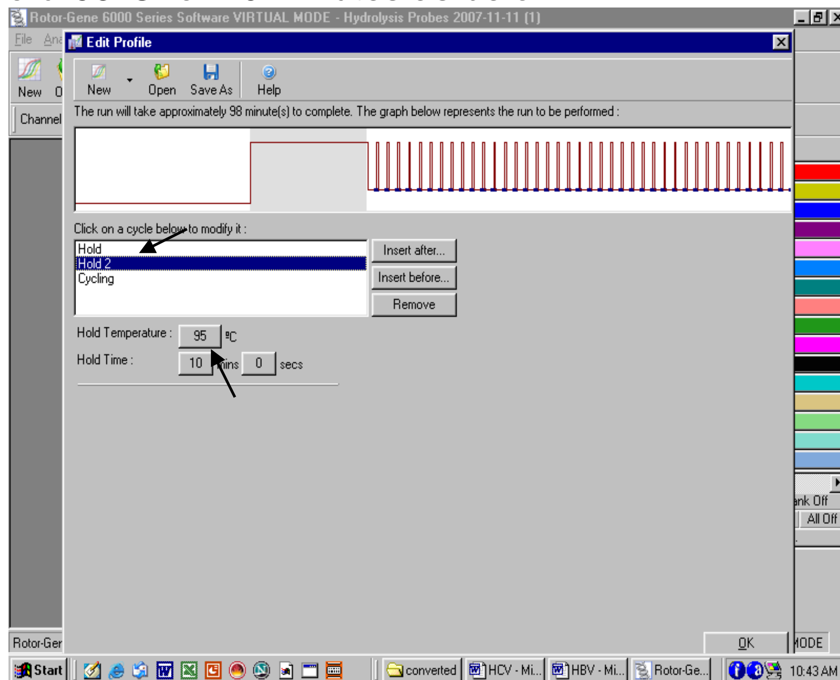


Fig. 27. Initial activation of the Hot Start enzyme. Generally the window will open with the Hold Temp as 95°C and the Hold Time as 10 minutes. In case it is different then set the same to as shown above.

j) Setting the Cycling and acquisition.

When clicked on Cycling the window will open as below.

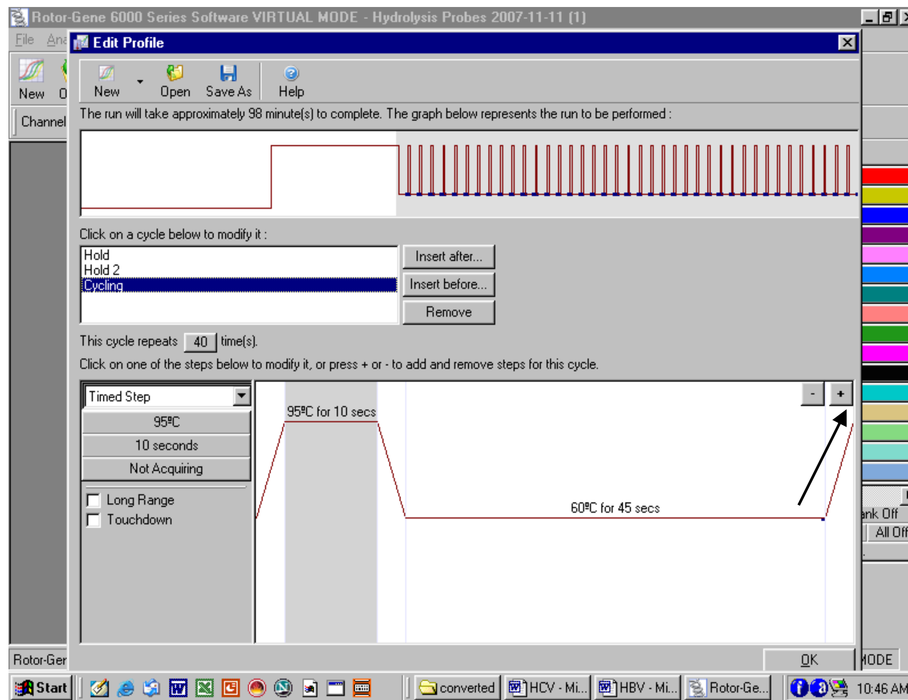


Fig. 28.

Click on the Plus sign at the right hand as shown in the figure to make the Cycling a three step process. A new window as shown below will be there.

Setting up of denaturation step in the cycling profile as depicted below i.e. 95°C for 15 seconds.

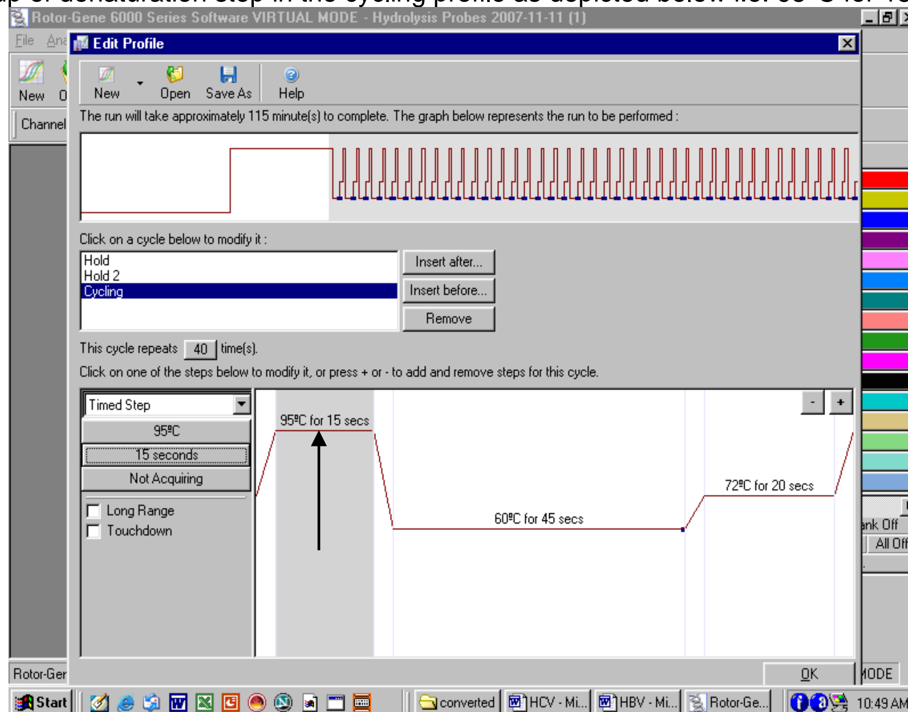


Fig. 29.

Setting up of Anneling step in the cycling profile as depicted below i.e. 55°C for 20 Seconds

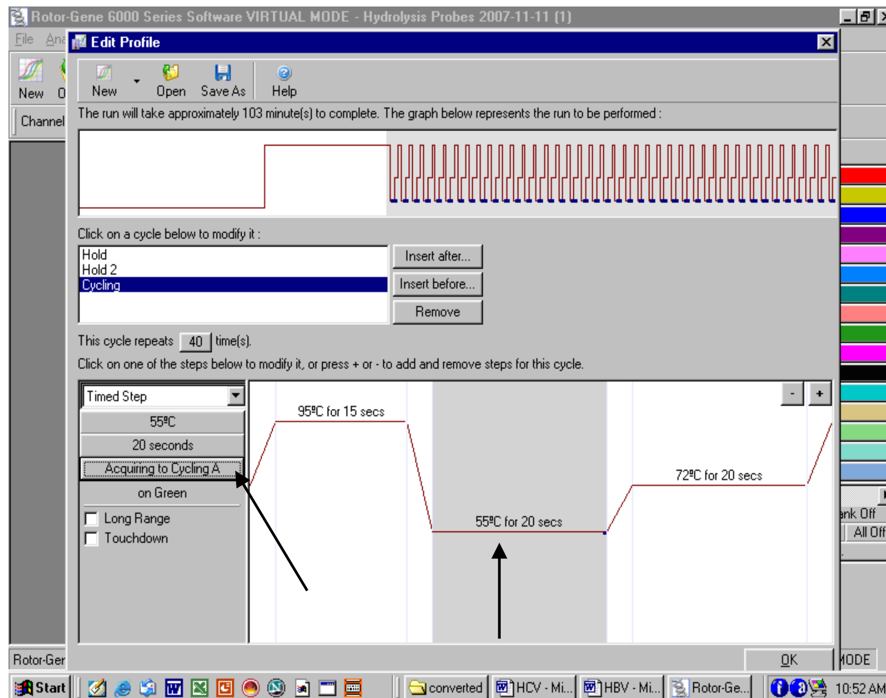


Fig. 30.

After setting the Anneling temperature and the time for anneling click on the "Acquiring to Cycling A" as shown by arrow. A New window will open as shown below.

Defining the Data acquiring channel i.e Green (FAM) & Yellow (JOE)

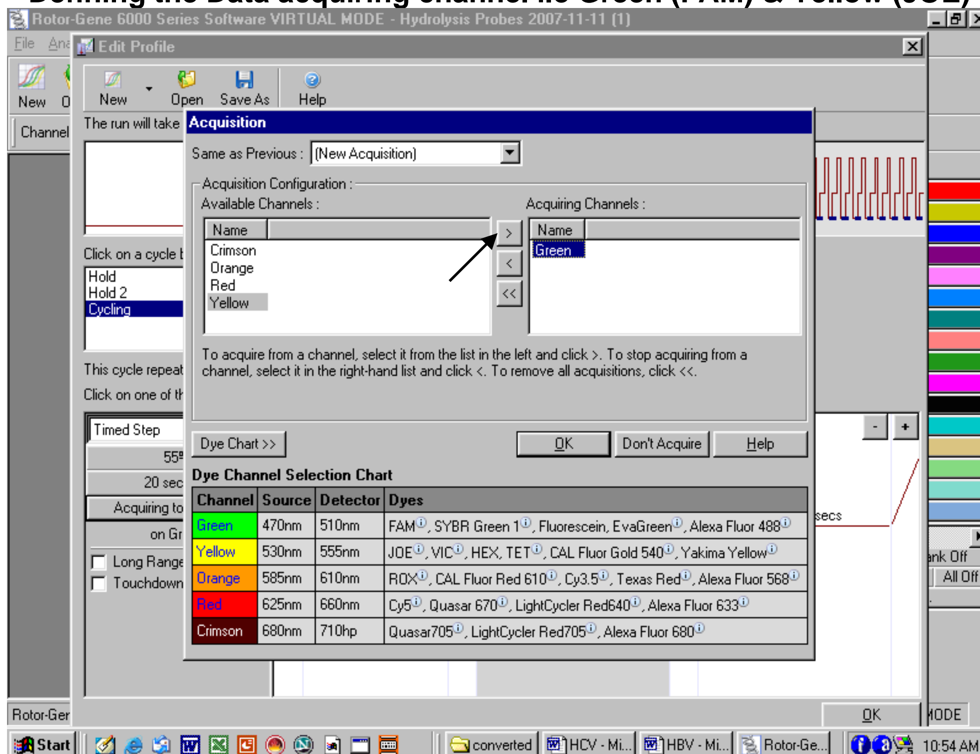


Fig. 31.

Highlight the Yellow and then press the right arrow. Just see before shifting the yellow to right that there is no other channel in the right except for Green. In case any other Channel appears besides Green on the right then the same be shifted to the left.

Confirmation of Channels as shown below.

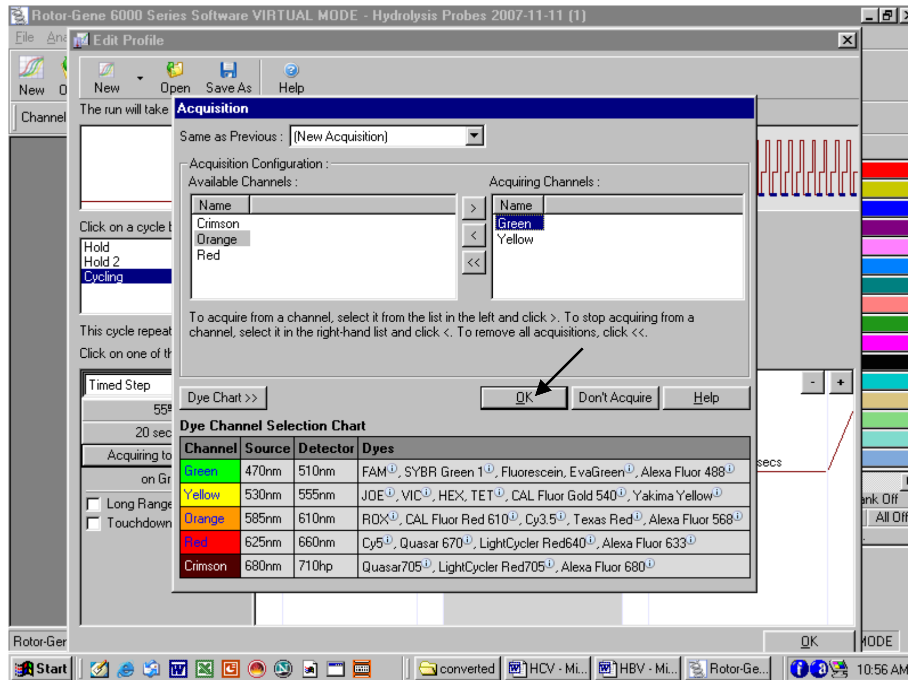


Fig. 32.

Once the Yellow and Green Channels are on the Right side then press OK as shown by the arrow.

Setting up of Extension step in the cycling profile as depicted below i.e. 72°C for 15 Seconds

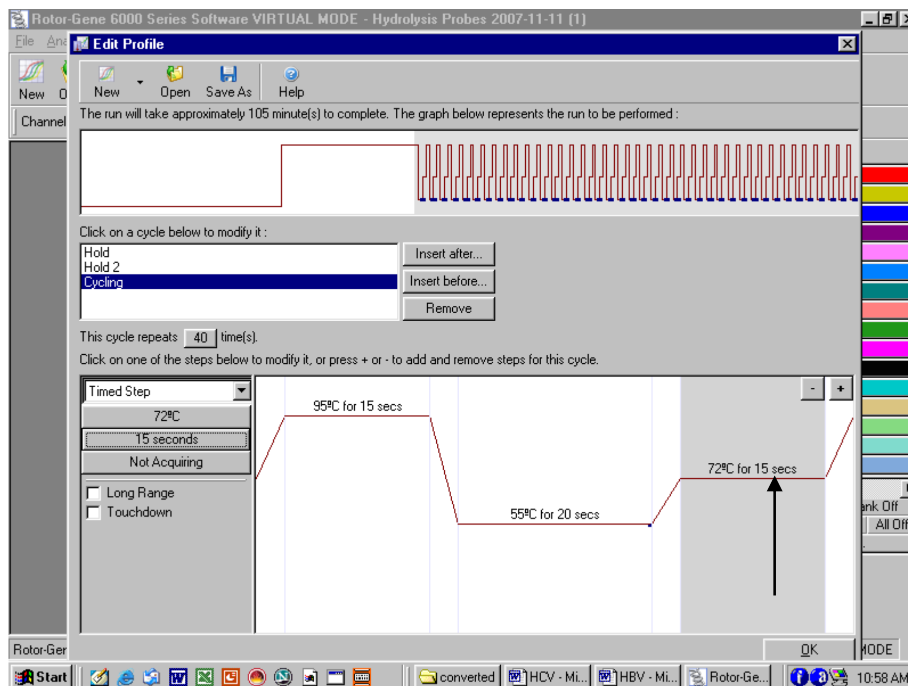


Fig. 33.

Setting up of Number of Cycles to 45 cycles in the cycling profile as depicted below.

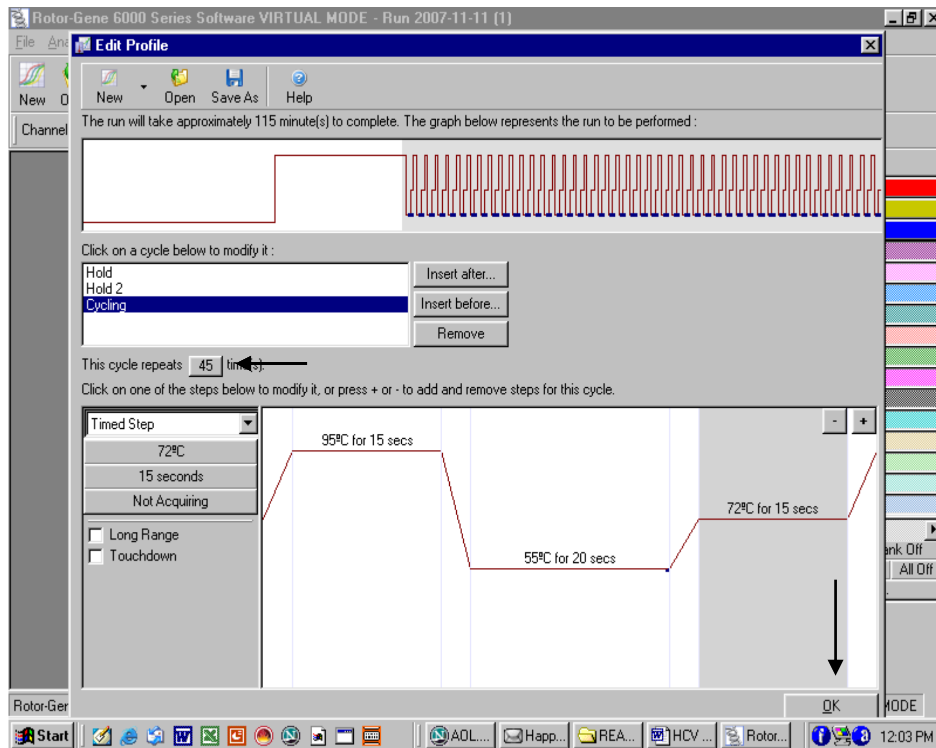


Fig. 34.

After setting the number of Cycles Press OK.

k) Setting the gains for the acquiring channel by clicking at the Gain Optimisation button as shown below.

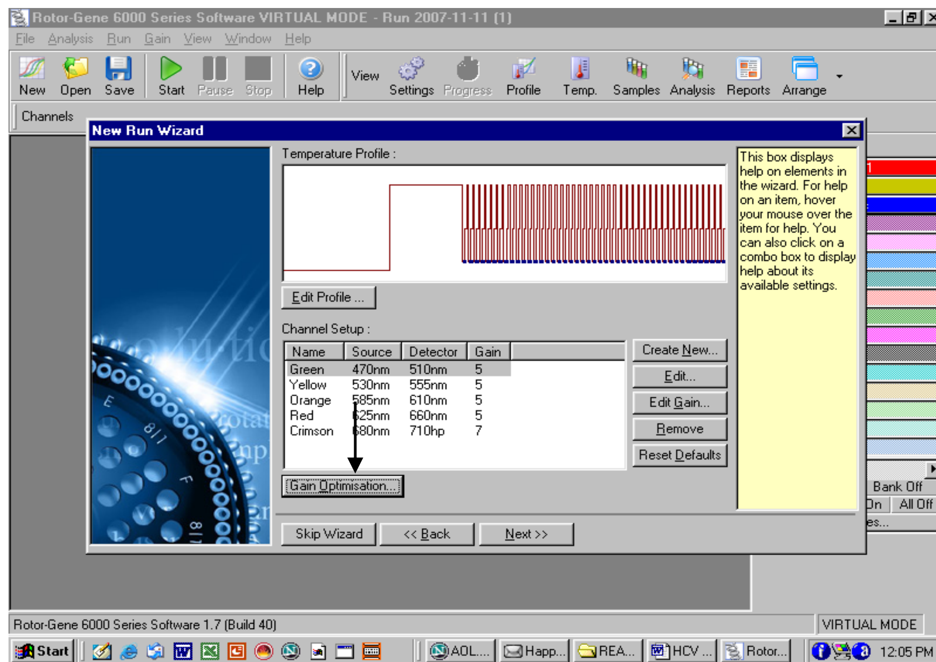


Fig. 35

The detection range of the fluorescence channels has to be determined according to the fluorescence intensities in the PCR tubes. This adjustment is done in the menu window *Auto Gain optimization Setup* (activation in menu window *New Experiment Wizard* under Gain Optimization). Please set the temperature to the annealing temperature of the amplification program (compare Fig.36 .

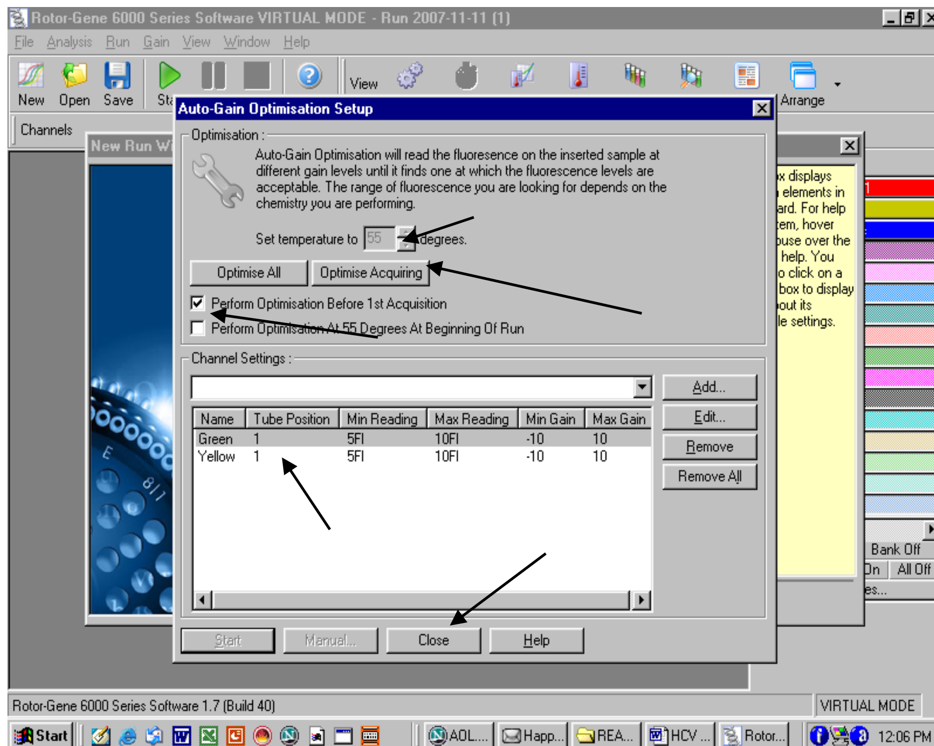


Fig. 36.

The following needs to be done.

- Click on the Optimise Acquiring
- Click on Set temperature To Adjust the Temperature to 55°C.
- Click on the Box Perform Optimisation before 1st Acquisition.
- Just see that below the channel settings there appear only two channels i.e. Green and Yellow. In case it is different then the channels have not been set properly. Close the window and reset the channels.
- Then Press Close.
- The press Next as shown below.

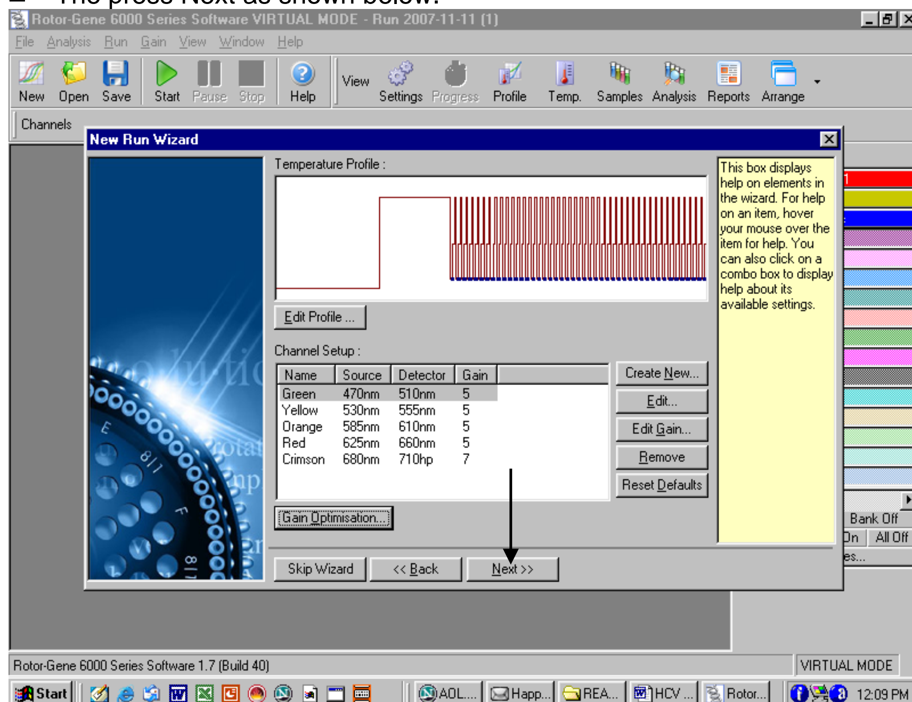


Fig. 37.

L) PRESS Start RUN

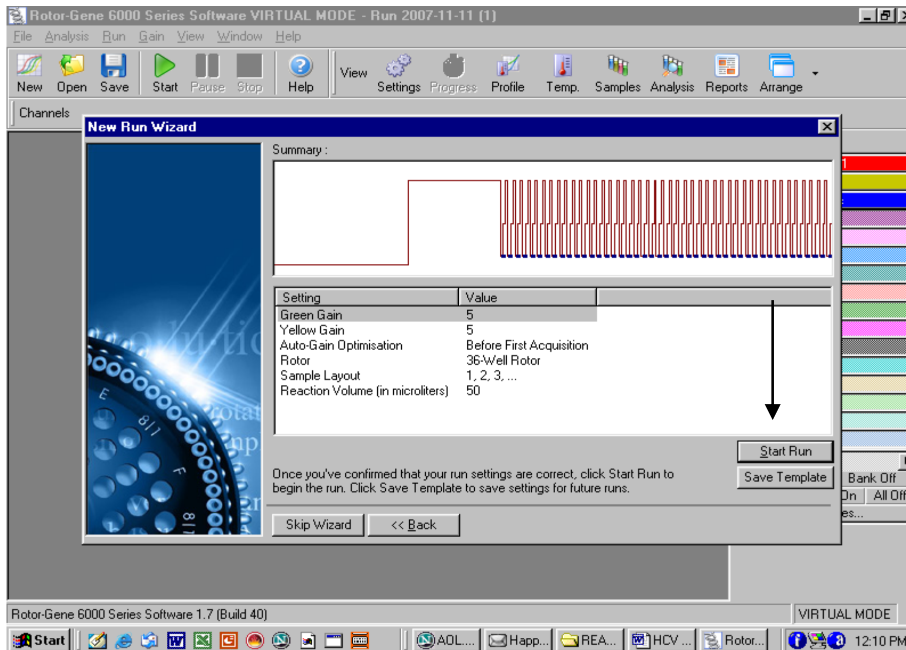


Fig. 38.

Saving the RUN File.

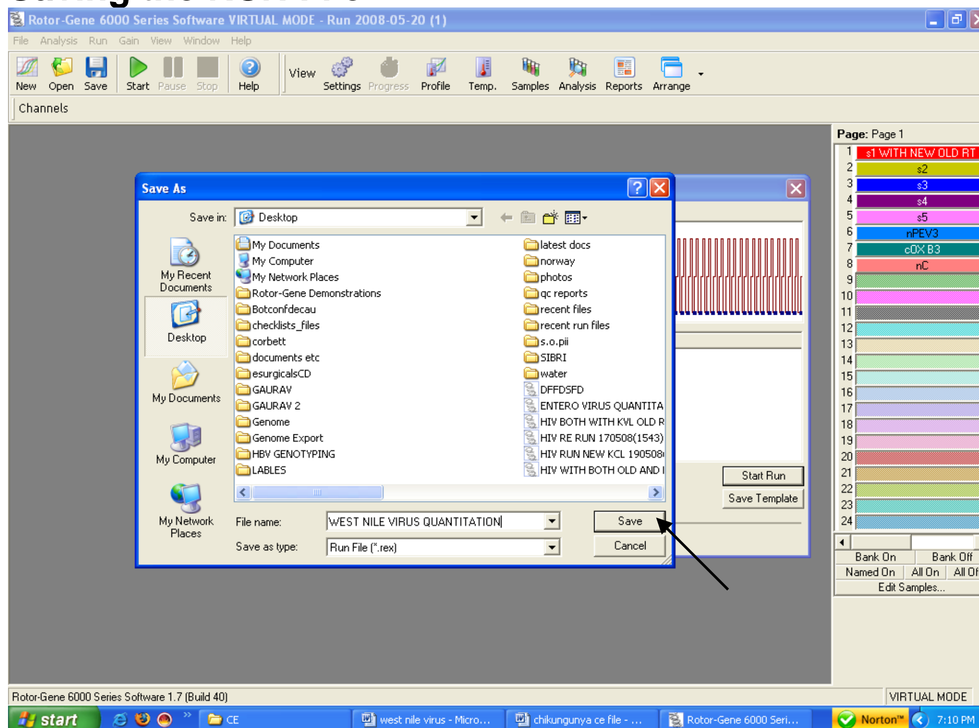


Fig. 39.

Store the run file either in my documents or a designated folder as shown above.

The moment save button is clicked after the file name, machine will start.

9. Generated Data Interpretation & Analysis

Data analysis is performed with the *RotorGene*™ software according to the manufacturer's instructions (*RotorGene*™ *6000 Operator's* Manual).

The following results are possible:

A signal is detected in fluorescence channel Cycling A.Green.

The result of the analysis is positive: The sample contains WEST NILE VIRUS RNA.

In fluorescence channel Cycling A.Green no signal is detected.

No WEST NILE VIRUS RNA detectable. It can be considered negative if there is amplification in the Yellow Channel. Please refer for details on PCR inhibition in the section PCR inhibition.

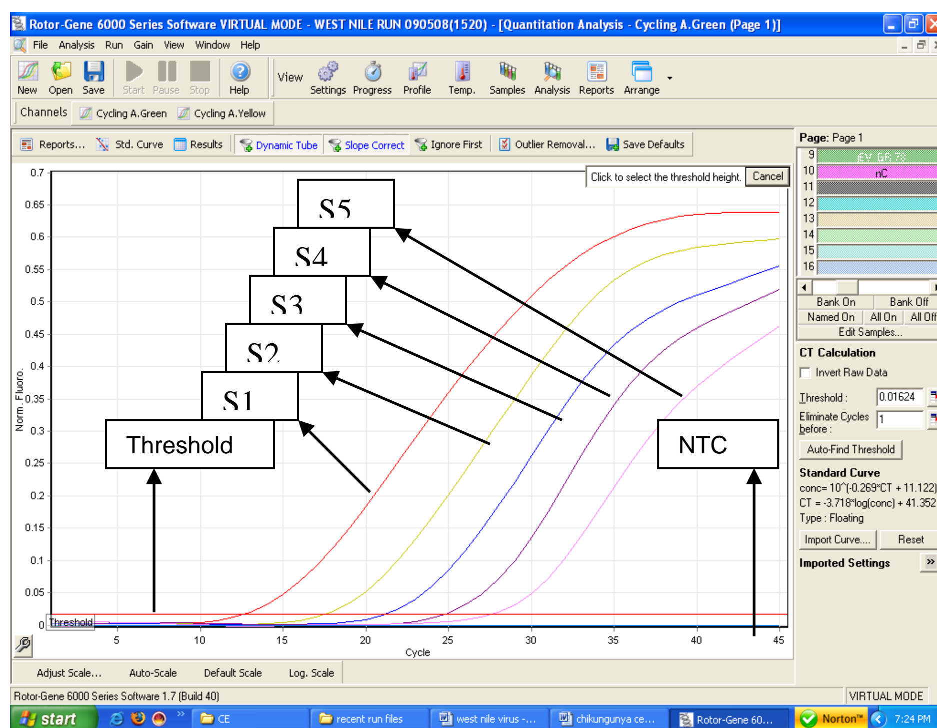


Fig. 42: Detection of the quantitation standards *(WNV S 1-5)* in fluorescence channel Cycling A.Green. NTC: non-template control.

Examples of positive and negative PCR reactions are given in the above figure.

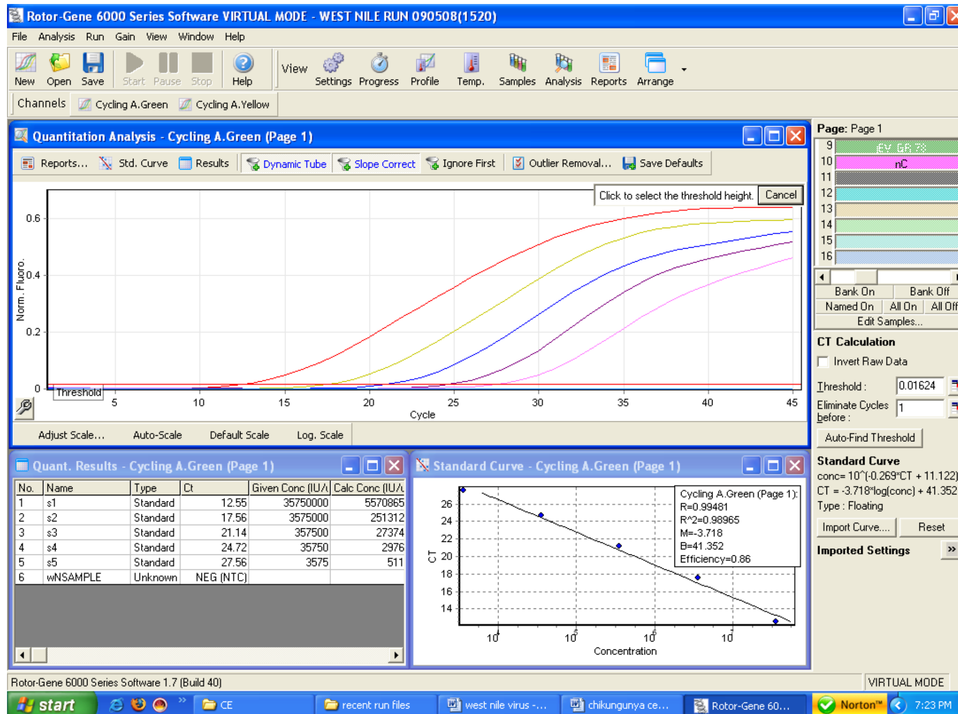


Fig. 43.

Example of analysed data for WEST NILE VIRUS where all the amplification curves can be seen as well as The standard curve, reaction efficiency, M value etc. can be seen and the quantitative data for the samples can be seen.

Inhibition Control gene amplification.

Analyze the Yellow Channel

PCR Inhibition

A signal is detected in fluorescence channel Cycling A. Yellow: **No PCR inhibition**

In fluorescence channel Cycling A. Yellow no signal is detected.

But signal detected in Green: The sample is positive for WEST NILE VIRUS RNA.

In fluorescence channel Cycling A. Yellow no signal is detected.

No signal detected in Green as well: A possible PCR inhibition has occurred.

10. Troubleshooting

1. No signal with positive Standards (WNV *S 1-5*) in fluorescence channel Cycling A.Green.

- Incorrect programming of the *Rotor-Gene*™ 6*000.*

➔ Repeat the PCR with corrected settings.

2. Weak or no signal in fluorescence channel Cycling A. Green:

- The PCR conditions do not comply with the protocol.

  ➔ Repeat the PCR with corrected settings.

- The WEST NILE VIRUS Super Mix *R1* has been thawed and frozen too often.
- The WEST NILE VIRUS Super Mix R1 has been kept at +4°C for longer than 5 hours.

  ➔ Please mind the storage conditions given in the **Storage.**

  ➔ Repeat the assay using a new WEST NILE VIRUS super mix (R1).

- **The PCR was inhibited.**

  ➔ Make sure that you use a recommended extraction method (see **8.a. RNA extraction**) and stick closely to the manufacturer's instructions.

11. Specifications

11.a Sensitivity and Reproducibility

In order to determine the sensitivity of the ***Geno-Sen's*** *WEST NILE VIRUS Real Time PCR Kit*, a dilution series has been set up from 10^6 down to 10^0 Copies/µl of WEST NILE VIRUS RNA and analyzed with the ***Geno-Sen's*** *West Nile Virus Real Time PCR Kit*. The assays were carried out on three different days in the form of 8-fold determinations. The results were determined by a probit analysis. The detection limit as determined for ***Geno-Sen's*** *West Nile Virus Real Time PCR Kit* is consistently 80 copies/ml. This means that there is 95% probability that 80 copies/ml will be detected.

Analytical Sensitivity

Analytical Sensitivity in Conjunction with the ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) of the** ***Geno Sen's®*** *WEST NILE Real Time PCR RG Kit on ROTOR GRNR 3000/6000 was determined by Spiking a known negative Serum to a nominal 90 copies/ml. This was subjected to extraction using the* ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) eight times with starting volume of 150**µl & elution volume of 60µl.

All the Eight extractions were then analyzed with the ***Geno Sen's®*** WEST NILE *Real Time PCR RG Kit along with the standards & the NTC.*

All the eight extractions were amplified correctly & the mean value obtained for the eight replicates was 89 copies/ml.

Hence Analytical Sensitivity in Conjunction with the ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001) of the** ***Geno Sen's®*** WEST NILE *Real Time PCR RG was determined to be 90 copies/ml.*

11.b Specificity

In order to check the specificity of the ***Geno-Sen's*** WEST NILE VIRUS Real Time PCR kit, different RNA & DNA listed below were analyzed with ***Geno-Sen's*** WEST NILE VIRUS Real Time PCR Kit. None of these led to a positive signal with the ***Geno-Sen's*** WEST NILE VIRUS Real Time PCR kit. Gene sequence analysis of the amplified region of WEST NILE VIRUS shows a pronounced homology among the various WEST NILE VIRUS subtypes, and no homology with other RNA. Besides which utmost care has been taken in selection of the primers & probes being used in the kit.

To further Validate the stringent data In order to check the specificity of the ***Geno Sen's®*** WEST NILE *Real Time PCR RG Kit*, different RNA & DNA listed below were analyzed with ***Geno Sen's®*** WEST NILE *Real Time PCR RG Kit*. None of these led to a positive signal with the ***Geno Sen's®*** WEST NILE *Real Time PCR RG Kit*

Vericella Zoster Virus	Hepatitis B Virus	N. Meningitis
Human Herpes Virus 1 & 2	Hepatitis A Virus	S. Pneumonia
Epstein barr Virus	Hepatitis E Virus	JEV
Cytomagalovirus	HIV-1	Mycobacterium tuberculosis
Chlamydia pneumonia	HIV 2	Chickungunya Virus
Parvovirus B 19	EnteroVirus	Staphylococcus aureus
Dengue Virus 1-4	H. Influenza	Salmonella enteritidis
Leprosy	Malaria	Scrub typhus
B.pseudomallie	Hepatitis C Virus	Leptospira interrogans.

In order to validate the Specificity further with wet lab testing the following Known clinical samples were analyzed using the ***Geno Sen's®*** WEST NILE Real Time PCR RG kit on ROTOR GENE 3000/6000 machine.The extraction was carried out with the ***Geno Sen's®*** **Viral RNA Extraction Mini Kit — for RNA purification (Cat . No. 98001)**

The run was carried out with the known set of standards in order to quantitate the WEST NILE RNA Gene.

Sample Type	Bronchial swab	Plasma	CSF
High +ve's	7	2	0
Medium +ve's	11	4	3
Low +ve's	9	4	3
Extremely low +ve's	4	2	0
Negative samples.	6	4	5
	37	16	11

All the above samples were correctly identified by the ***Geno Sen's***® WEST NILE Real Time PCR RG kit & all the 6 extremely low samples were accurately detected by the ***Geno Sen's***® WEST NILE Real Time PCR RG kit & exhibited copies around 90 copies /ml or less than 90 copies/ml.

Further studies are underway on this aspect.

12. Warranty::

Products are guaranteed to confirm to the quality and content indicated on each vial and external labels during their shelf life. Genome Diagnostics Pvt. Ltd. obligation and purchaser's rights under the warranty are limited to the replacement by Genome Diagnostics Pvt. Ltd of any product that is shown defective in fabrication, and that must be returned to Genome Diagnostics Pvt. Ltd Freight prepaid, or at Genome Diagnostics Pvt. Ltd. option, replacement of the purchasing price. Any complaint on damaged goods during transport must be directed to the handling or transport agent.

In Vitro Diagnostic Medical device, as per the Directive 98/79/EC specifications. This product must be used by qualified professionals only. It is the responsibility of the user to ascertain that a given product is adequate for a given application. Any product not fulfilling the specifications included in the product sheet will be replaced. This warranty limits our responsibility to the replacement of the product. No other warranties, of any kind, express or implied, including without limitation, implicit warranties of commercialization ability or adequacy for a given purpose, are provided by Genome Diagnostics Pvt. Ltd. Genome Diagnostics Pvt. Ltd will not be responsible for any direct, indirect, consequential or incidental damage resulting of the use, misuses, results of the use or inability to use any product.

13. Limitations of product use:

a.) All reagents may exclusively be used for *in vitro* diagnostics.
b.) The product is to be used by personnel specially instructed and trained in for the *in-vitro* diagnostics procedures only.
c.) It is important to pipet the indicated quantities, and mix well after each reagent addition. Check pipettes regularly.

d.) Instructions must be followed Correctly in order to obtain correct results. If the user has any questions, please contact our Technical Dept. (dharam.vsnl@gmail.com OR genome24@rediffmail.com).
e.) This test has been validated for use with the reagents provided in the kit. The use of other Reagents or methods, or the use of equipment not fulfilling the specifications, may render equivocal results. User is responsible for any modifications done in any of the indicated parameters. Compliance with the kit protocol is required.
f.) Detection of Viral RNA depends on the number of Viruses present in the sample, and can be affected by sample collection methods, patient-related factors (e.g. age, symptoms), or for infection stage and sample size.
g.) False negative results may be obtained due to polymerase inhibition. It is recommended to perform control reactions to distinguish between inhibition and true negatives. This is achieved by the inhibition Control included in the kit.
h.) Cross contamination between samples and exogenous DNA can only be avoided by following good laboratory practice. Instructions in this document must be strictly followed.
i.) Attention should be paid to expiration dates printed on the kit box and labels of all components. Do not use expired components.

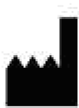
If you have any further questions or problems, please contact our technical support at dharam.vsnl@gmail.com OR genome24@rediffmail.com

14. LIST OF GENO-SEN'S RANGE OF REAL TIME PCR KITS

S.NO.	PRODUCT
1	**HIV-1 RG quantitative Real time PCR kit.**
2	**HBV RG quantitative Real time PCR kit.**
3	**HCV RG quantitative Real time PCR kit.**
4	**HCV Genotyping 1/2/3/4 RG qualitative Real time PCR kit.**
5	**HEV RG quantitative Real time PCR kit.**
6	**HAV RG quantitative Real time PCR kit.**
7	**JEV RG quantitative Real time PCR kit.**
8	**ENTEROVIRUS RG quantitative Real time PCR kit.**
9	**DENGUE RG quantitative Real time PCR KIT**
10	**HSV 1 & 2 RG quantitative Real time PCR kit.**

11	**CMV RG quantitative Real time PCR kit.**
12	**West Nile Virus RG quantitative Real time PCR kit.**
13	**Measles Virus RG quantitative Real time PCR kit.**
14	**West Nile Virus RG quantitative Real time PCR kit.**
15	**H5 N1 (Bird Flu) RG quantitative Real time PCR kit.**
16	**Chikungunya RG quantitative Real time PCR kit.**
17	**TTV RG quantitative Real time PCR kit.**
18	**SARS RG quantitative Real time PCR kit.**
19	**JC/BK Virus RG quantitative Real time PCR kit.**
20	**MTb Complex RG quantitative Real time PCR kit.**
21	**MTb Complex /MOTT RG quanlitative Real time PCR kit.**
22	**Chlamydia pneumonia RG quantitative Real time PCR kit.**
23	**Streptococcous pneumonia RG quantitative Real time PCR kit.**
24	**N. Meningitis RG quantitative Real time PCR kit.**
25	**H. Influenza RG quantitative Real time PCR kit.**
26	**Leprosy RG quantitative Real time PCR kit.**
27	**Helicobacter Pylori RG quantitative Real time PCR kit.**
28	**Scrub Typhus RG quantitative Real time PCR kit.**
29	**B. Pseudomalie RG quantitative Real time PCR kit.**
30	**Filaria RG quantitative Real time PCR kit.**
31	**Leptospira(pathogenic) RG quantitative Real time PCR kit.**
32	**CCL3-L1 RG quantitative Real time PCR kit.**
33	**Malaria (P. Vivax) RG quantitative Real time PCR kit.**
34	**Bcr/abl Major RG quantitative Real time PCR kit.**
35	**Bcr/abl Minor RG quantitative Real time PCR kit.**

36	**PML/RARA RG quantitative Real time PCR kit.**
37	**RARA/PML RG quantitative Real time PCR kit.**
38	**GAPDH RG quantitative Real time PCR kit.**
39	**β-Actin RG quantitative Real time PCR kit.**
40	**β-Globin RG quantitative Real time PCR kit.**
41	**Abl gene RG quantitative Real time PCR kit.**
42	**Rabies RG quantitative Real time PCR kit.**
43	**Factor V Leiden detection RG Real time PCR kit.**



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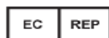

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