TRUPCR™

TRUPCR MTHFR QUALITATIVE KIT

MTHFR (C677T) Real time PCR Detection

3B BlackBio Biotech India Ltd
A joint venture of Biotools B&M Labs, Spain and Kilpest India Ltd.
7-C Industrial Area, Govindpura Bhopal-462023 (M.P.) India
Phone: +91-755-4076518; 4077847 Fax: +91-755-2580438
Website: www.3bblackbio.com E-mail: info@3bblackbio.com

CE IVD

TRUPCR MTHFR QUALITATIVE KIT

Detection of *MTHFR Mutation* in clinical samples

CE IVD

REF Product No.: 3B1331/3B1332

48/96 tests

Temperature limitation

June 2018

Manufactured by- **3B BlackBio Biotech India Ltd.**

7-C Industrial Area, Govindpura, Bhopal-462023 (M.P)

EC	REP

Wellkang Ltd,
16 Castle St, Dover, CT16 1PW, UK;
Black Church, St. Mary's Place,
Dublin 7, Ireland

CONTENT	PAGE NO.

INTENDED USE

The **TRUPCR MTHFR Qualitative Kit** is designed for the qualitative detection of MTHFR Mutation (C677T) in human blood. The results from the TRUPCR MTHFR qualitative Kit must be interpreted within the context of all relevant clinical and laboratory findings.

MTHFR MUTATION

The Methylenetetrahydrofolate Reductase (MTHFR) gene codes for the enzyme that catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the major circulatory form of folate in the body. The various functions of 5- methyltetrahydrofolate include participation in many complex biochemical pathways such as nucleotide synthesis, DNA methylation, methylation of proteins, neurotransmitters and phospholipids and also acting as a cofactor in the remethylation of Homocysteine to Methionine[1] Some rare mutations can cause severe MTHFR deficiency, which may lead to homocysteinemia and severe clinical symptoms, like thrombosis and impairment of psychomotor development[2].

Homocysteine is an important substance in the blood as elevated levels of Homocysteine has been found to be the causative agent of various diseases such as; Cerebrovascular disease cerebral vein thrombosis, coronary artery disease, myocardial infarction, venous thrombosis neural tube defects leading to dementia and Alzheimer's disease osteoporosis, diabetes, complications in pregnancy, etc[3].

Genetic factors involve point mutations in the MTHFR gene. The MTHFR gene is roughly 19300bp long and is located on the short arm of the 1st pair of chromosomes at position 36.3. The cDNA sequence is 2.2 kilo bases in length and consists of 11 exons. Alternative splicing of the gene is observed in humans. The major product of the MTHFR gene in humans is a 77-kDa protein[4].

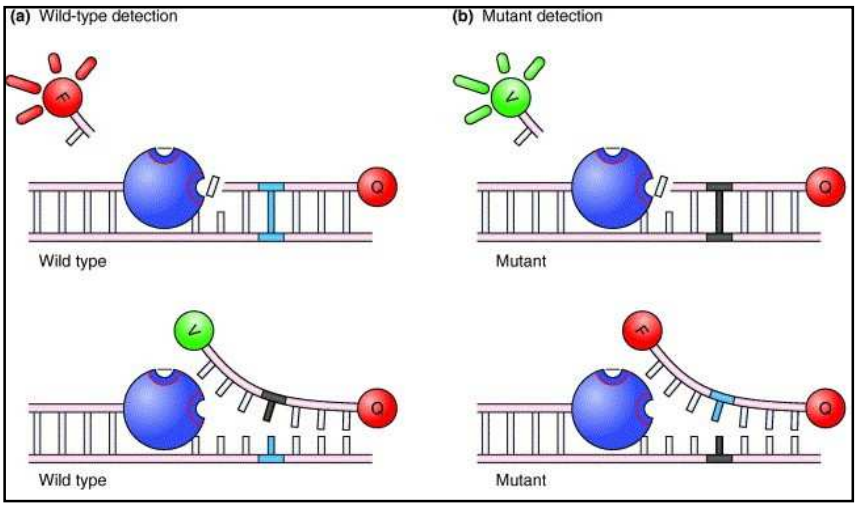
A genetic polymorphism commonly associated with severe MTHFR deficiency is defined by a C to T substitution (cytosine to thymine) at position 677 (C677T) of the MTHFR gene, which leads to the incorporation of amino acid alanine (A) instead of valine (V) at position 222 of the MTHFR protein. The altered MTHFR is known as "thermolabile MTHFR". Homozygous and heterozygous carriers of this mutation both show reduced MTHFR activity. In particular, homozygous carriers suffer from significantly increased blood levels of homocysteine. In general, increased homocysteine levels are considered a risk factor of vascular diseases (e.g. arterial thrombosis)[5].

PRINCIPLE

TRUPCR MTHFR Qualitative Kit is an allelic discrimination real-time polymerase chain reaction (PCR) assay for qualitative detection of C677T mutation against a background of wild-type genomic DNA.

In an allelic discrimination, two different probes specific for each allele are included in the PCR assay. Each probe is labeled with a different fluorescent dye (such as FAM or HEX/VIC) at its 5' end and contains a non fluorescent quencher at the 3' end. During qPCR amplification of the target DNA the probes will compete for binding across the variant region. The probe that is 100% homologous to the DNA binding site will preferentially bind and give a fluorescent signal as PCR proceeds. It follows that the wild-type sequence will give a strong amplification plot through one channel whilst giving a very weak signal through the alternative channel. Homozygous variant samples will give an exactly inverse result. A mismatch between probe and target greatly reduces the efficiency of probe hybridization and cleavage and no reporter dye is released. Thus, substantial increase in FAM or HEX/VIC dye fluorescence indicates homozygosity for the FAM- or HEX/VIC- specific allele. An increase in both signals indicates heterozygosity.

In this kit there are two independent reactions running in parallel in each tube: the first detects MTHFR mutant gene (HEX channel), second detects MTHFR wild type gene (FAM channel).



REAGENTS

The Kit contains amplification reagents for performance of 48/96 amplification reactions. Thaw and handle reagents on ice. Do not freeze/thaw Kit vials repeatedly. In case of frequent use, we recommend to aliquot the contents of the vials into 10 reactions each. This will also rule-out kit/ reagent contamination.

TRUPCR MTHFR QUALITATIVE KIT

Reagent	Description	Volume in µL 48 reactions	Volume in µL 96 reactions
Multiplex Master Mix	• Hot-start DNA polymerase • Reaction Buffer • dNTPs (dATP, dCTP, dGTP, dTTP) $MgCl_2$ and stabilizers	240 µL X 2	240 µL X 4
MTHFR Primer probe mix	• Primer and probe mix for MTHFR (C677T) mutation detection	120 µL X 2	120 µL X 4
Homozygous Wild Type Control	• Wild Type Positive Control MTHFR Mutation	25 µL X 2	50 µL X 2
Heterozygous Cut Off Control	• Heterozygous Mutant Positive Control MTHFR Mutation	25 µL X 2	50 µL X 2
Homozygous Mutant Control	• Homozygous Mutant Positive Control MTHFR Mutation	25 µL X 2	50 µL X 4
Negative Control	• Sterilized water	250 x 2 µL	250 x 4 µL

DNA EXTRACTION

Sample collection should follow all the usual sterility precautions. Blood must be treated with EDTA. Other anticoagulation agents, as heparin, are strong inhibitors of TAQ polymerase and so they could alter the efficiency of the amplification reaction. Fresh blood can be stored at +2/+8°C if processed in a short time.

DNA Extraction kits are available from various manufacturers (Table below). Sample volumes for DNA Extraction procedure depend on the protocol used. The procedure for extraction should be carried out according to the manufacturer's instruction.

DNA quantity is determined by measuring optical density at 260 nm. DNA quality should be assessed by spectrophotometry or gel electrophoresis.

The A260/A280ratio should be 1.7–1.9. Smaller ratios usually indicate contamination by protein or organic chemicals. The qPCR reaction is optimized for 200 ng of purified genomic DNA.

Sample Material	Nucleic Acid Isolation Kit	Cat No.
Whole blood	TRUPCR BLLOD DNA Extraction Kit	3B205
	QIAamp DNeasy Blood &Tissue Kit	69581

TRUPCR BLOOD DNA EXTRACTION KIT

DNA EXTRACTION KIT	48 Reactions	96 Reactions
Reagent	**Volume**	**Volume**
Lysis Buffer BB3	15 ml	30 ml
Wash Buffer BBW (Concentrate)	19 ml	19 ml x 2
Wash Buffer BB5 (Concentrate)	13 ml	13 ml X 2
Elution Buffer BBE	15 ml	30 ml
Proteinase K (Lyophilized)	30 mg	30 mg X 2
Proteinase Buffer	1.5ml	1.5 ml X 2
DNA Spin Columns	50	100
COLLECTION TUBES	50	100

PREPARATION OF WORKING SOLUTIONS

Wash Buffer BBW: Add the below indicated volume of ethanol (96 – 100 %) to Wash Buffer BBW Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer BBW at room temperature (18 – 25° C) for up to one year.

Format	Volume of BBW	Volume of Ethanol to be added
48 rxns	19ml	25 ml to each bottle

Wash Buffer BB5: Add the below indicated volume of ethanol (96 – 100 %) to Wash Buffer BB5 Concentrate. Mark the label of the bottle to indicate that ethanol was added. Store Wash Buffer BB5 at room temperature (18 – 25° C) for up to one year.

Format	Volume of BB5	Volume of Ethanol to be added
48 rxns	13 ml	30 ml to each bottle

Proteinase K: Add the below indicated volume of Proteinase Buffer (**PB)** to dissolve lyophilized Proteinase K. Proteinase K solution is stable at - 20 °C for up to 6 months.

Format	Qty. of Proteinase K	Volume of PB to be added
48 rxns	30 mg	1.35 ml to each vial

INSTRUCTIONS FOR DNA EXTRACTION

Step	Description	
1	**Sample Lysis:** Add 25µl Proteinase K to the ***200 µl blood*** and mix well. Add **250 µl** of lysis **Buffer BB3** to samples and **vortex** the mixture vigorously (10-20 s). Incubate samples at **56°C** for **15 min**. *The lysate should become brownish during incubation with buffer BB3. Increase incubation time with Proteinase K (up to 30 min) and vortex once or twice vigorously during incubation if processing older or clotted blood samples*	+ 25µl Proteinase K (Vortex) + 250 µl Buffer BB3 Vortex Incubate at 56°C for 15 min.
2	**ADJUST DNA BINDING CONDITIONS** Add **250 µl** of ethanol (96-100%) to each sample and **vortex** again and incubate for 5 min.	+ 250µl Ethanol Mix
3	**BIND DNA** Pipette the mixture from previous step into the Spin Column placed into a 2 ml collection tube. Centrifuge for 1 min at 10,000 rpm. Discard flow-through.	Load lysate into a column *1 min, 10,000* rpm
4	**WASH SILICA MEMBRANE** **1st Wash** Add **500 µl Buffer BBW**. Centrifuge **1 min at 10,000 rpm**. **Discard flow-through** and place the column back into the Collecting tube. **2nd Wash** Add **500 µl Buffer BB5**. Centrifuge **1 min at 10,000 rpm.** Discard collecting tube with flow-through.	+ 500 µl BUFFER BBW *1 min, 10,000rpm* + 500 µl BUFFER BB5 *1 min, 10,000rpm*
5	**DRY SILICA MEMBRANE** Place the column into a new collection tube and centrifuge for **3 min at 12,000rpm**. Residual ethanol is removed during this step.	*3 min, 12,000 rpm*
6	**ELUTE HIGHLY PURE DNA** Place the column in a new 1.5 ml microcentrifuge tube and add 30-50 µl **prewarmed elution Buffer BBE (70°C)**. Dispense buffer directly onto the silica membrane. Incubate at room temperature for 1 min. Centrifuge **1 min at 10,000 rpm.**	30-50 µl BUFFER BBE (70ºC) Incubate 1 min *1 min, 10,000rpm*

REAL TIME PCR PROTOCOL

1. REACTION PREPARATION

a) Prepare the PCR Mix as follows:

b)

Name of the Reagent	For "1" rxn.
Multiplex Master Mix	10 µl
MTHFR Primer probe mix	5 µl

c) Transfer **15 µl** of the above prepared PCR Master Mix in 0.2 ml PCR tubes and close the tubes.

d) For **15 µl** of above reaction mix, add up to **5 µl** of sample DNA.

(The OD 260/280 of the sample should be measured spectrophotometrically and should be between 1.7 and 2.0)

e) Add **5 µl** of provided positive control to PC tube and make up the volume to 2**0 µl** with sterile water

f) Add **5 µl** of sterile water to NC tube and make up the volume to **20 µl** with sterile water

2. PROGRAM SET UP

Define the following setting for Temperature Profile and Dye Acquisition

Step	Program name	Temperature, °C	Time	Dye Acquisition	Cycles
1	Initial Denaturation	94	10 min	-	1
2	Denaturation	94	15 sec	-	40
	Annealing/Extension	60	60 sec	YES	

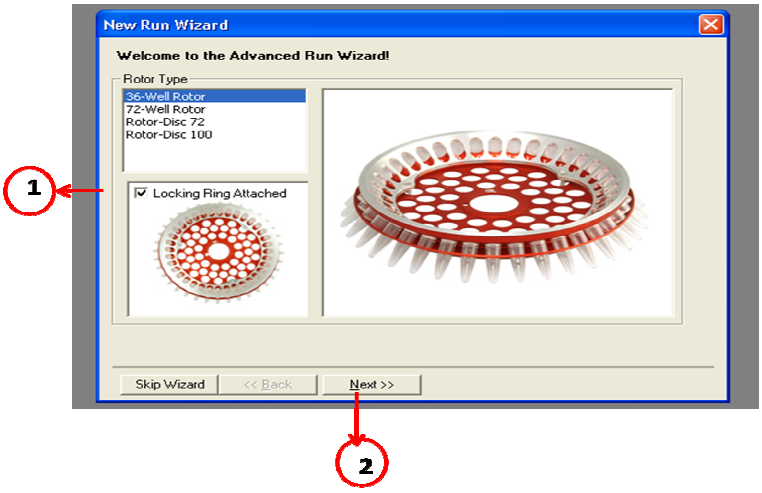
3. CHANNEL SELECTION

Define the following setting for channel selection

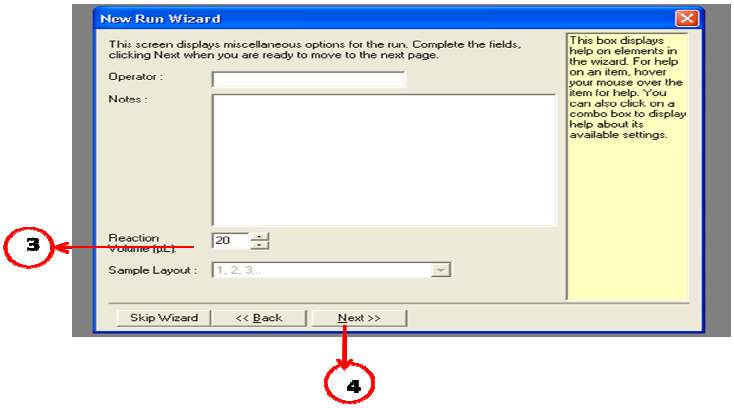
Detection	Detector Name	Reporter	Quencher	Gain Setup
MTHFR	MTHFR Mutant Type	HEX	None	Manual
MTHFR	MTHFR Wild Type	FAM	None	Manual

PROGRAM SET UP IN ROTOR-GENE Q

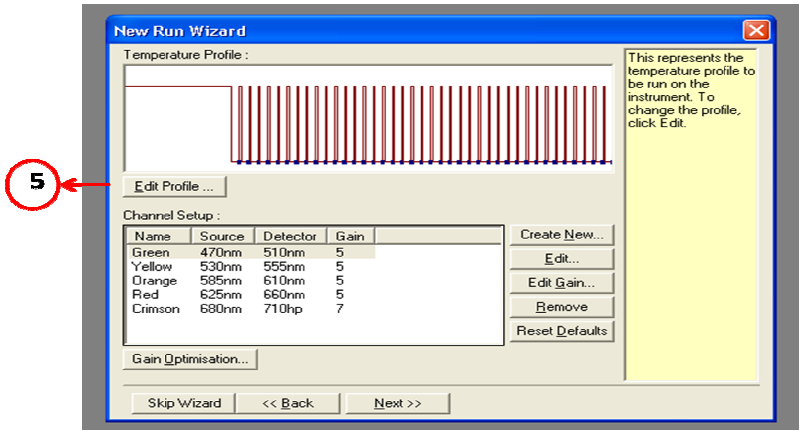
Open New RUN Wizard and select locking ring attached (1) and Click on Next (2)



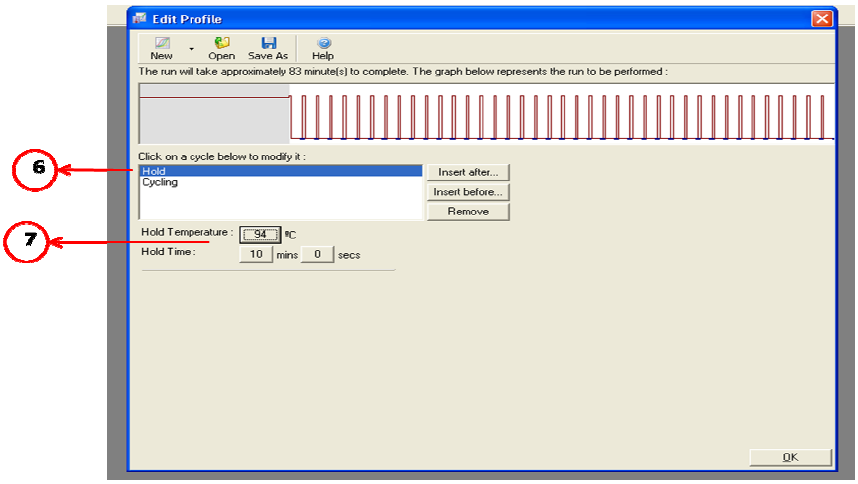
In the"New Run Wizard",set the volume to 20 µl (3)and click "Next"



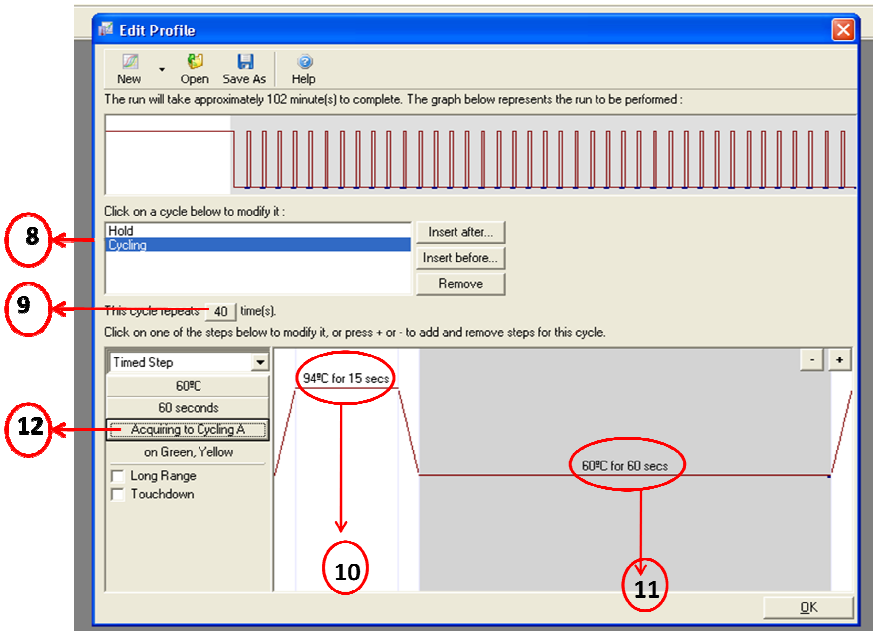
Click on Edit Profile (5) in New Run Wizard



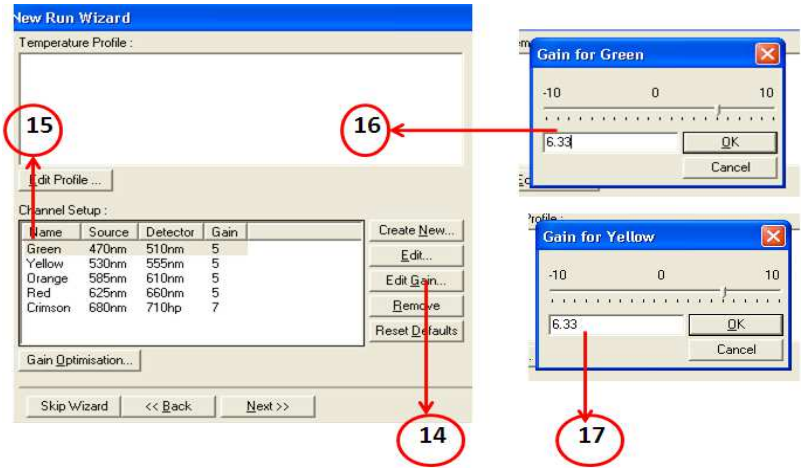
Click on Hold (6) and set temperature to 94 degree and time 10 mins (7)



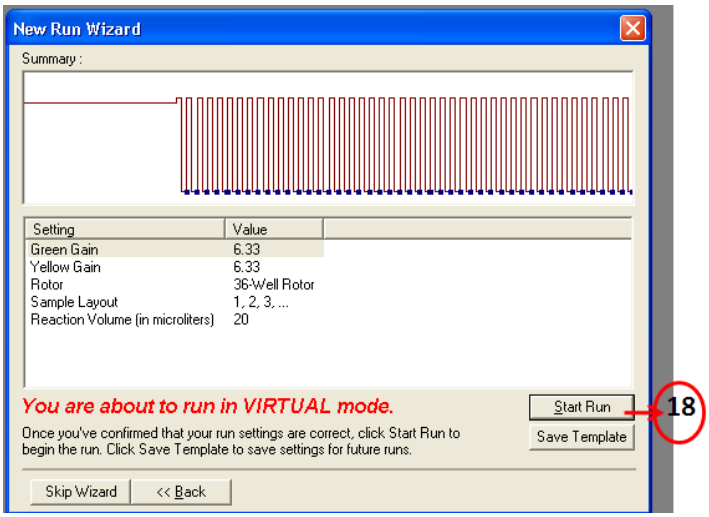
Click on Cycling (8), cycle repeat 40 (9), then cycling 94 degree for 15 sec (10), then enter 60 degree for 60 sec (11), then click on acquiring to cycling A (12) and select Green and Yellow Channel



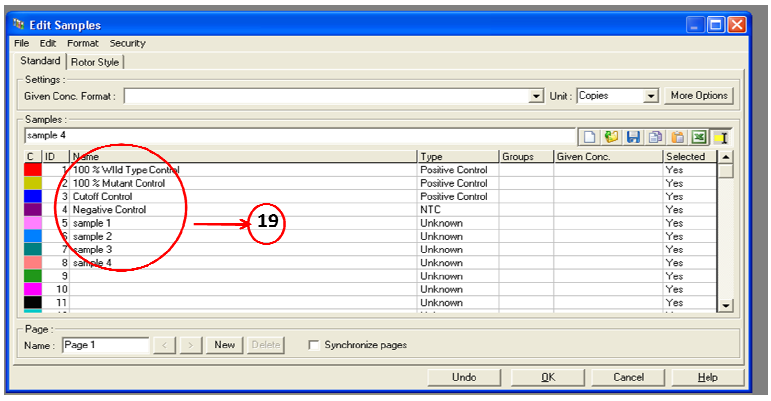
Click on Edit Gain (14), select Green and Yellow channel one by one (15), then keep optimized Gain for Yellow and Green (16) (17) (Example given for 6.33)



To start run click on Start Run (18)

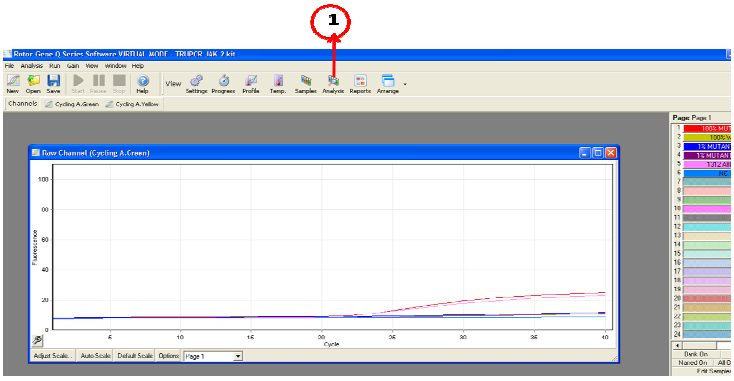


In Edit sample Fill sample details (19)

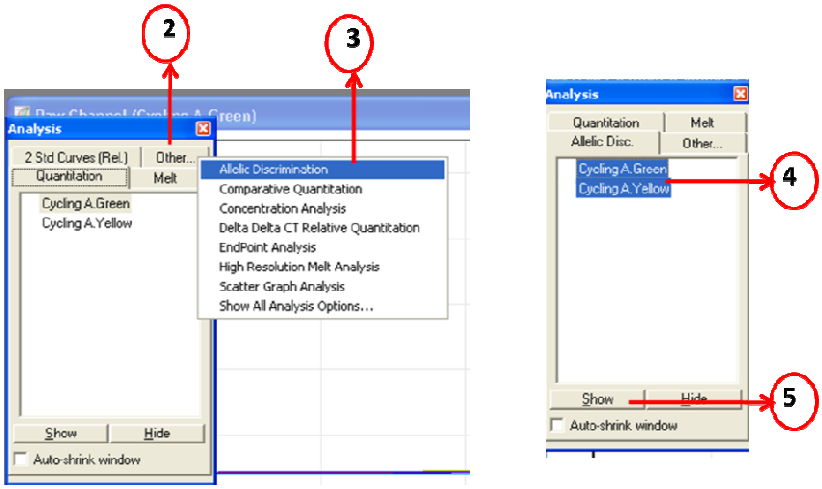


RESULT ANALYSIS

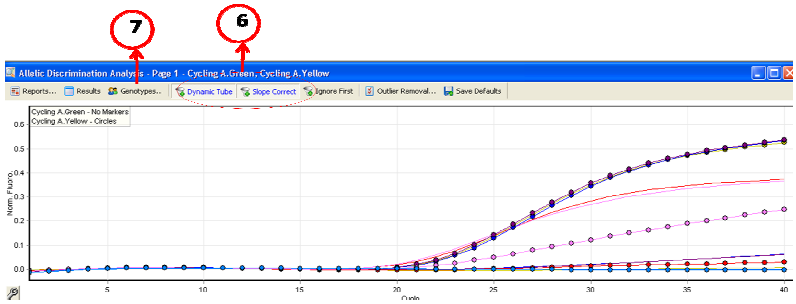
After completion of run, click on analysis (1)



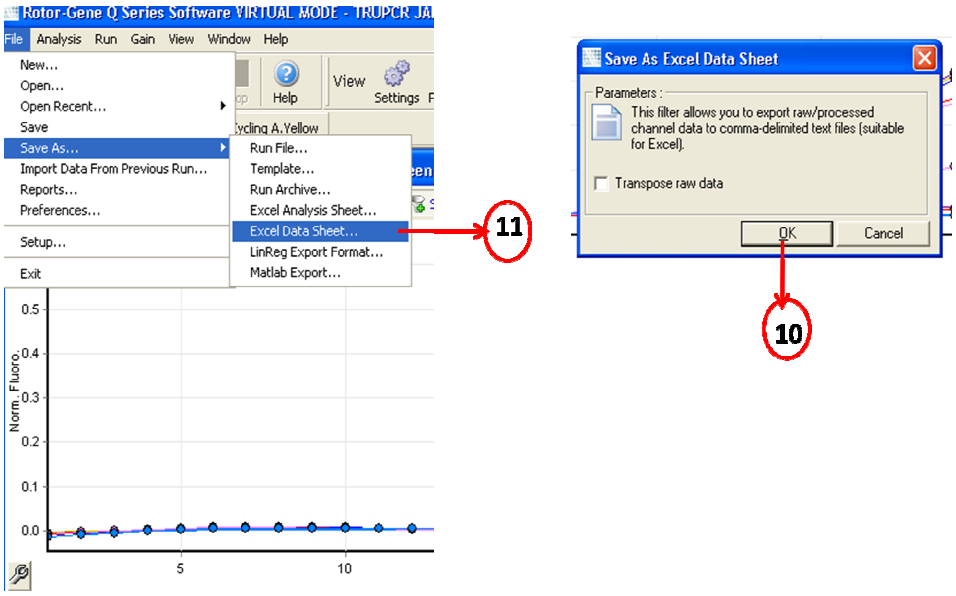
In Analysis window click on others (2), then click on Allelic discrimination (3), further select green and Yellow channel (4) and click on Show (5)



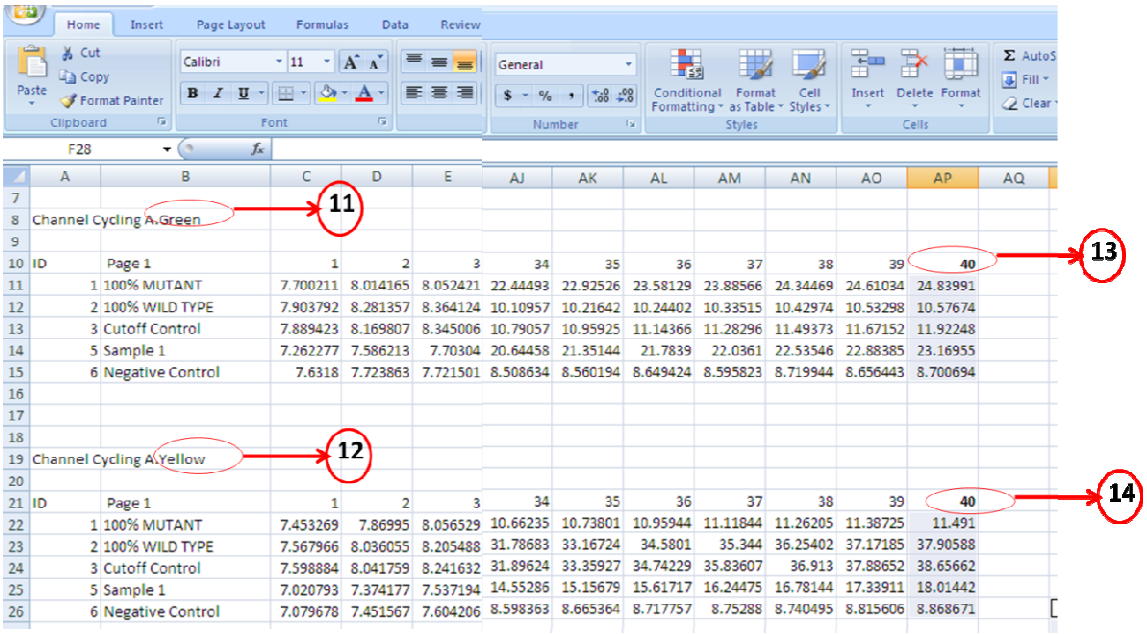
Click on Dynamic Tube and Slope correct (6) and then click on Genotype (7)



Save data by clicking on “File” then “Save as” then “Excel Data sheet” (11) then in popup window click OK



Open saved data and check for values for Green (11) and Yellow (12) and take the value of each channels in 40th Cycle



	A	B	C	D	E	AJ	AK	AL	AM	AN	AO	AP	AQ
7													
8	Channel Cycling A.Green												
9													
10	ID	Page 1	1	2	3	34	35	36	37	38	39	40	
11	1	100% MUTANT	7.700211	8.014165	8.052421	22.44493	22.92526	23.58129	23.88566	24.34469	24.61034	24.83991	
12	2	100% WILD TYPE	7.903792	8.281357	8.364124	10.10957	10.21642	10.24402	10.33515	10.42974	10.53298	10.57674	
13	3	Cutoff Control	7.889423	8.169807	8.345006	10.79057	10.95925	11.14366	11.28296	11.49373	11.67152	11.92248	
14	5	Sample 1	7.262277	7.586213	7.70304	20.64458	21.35144	21.7839	22.0361	22.53546	22.88385	23.16955	
15	6	Negative Control	7.6318	7.723863	7.721501	8.508634	8.560194	8.649424	8.595823	8.719944	8.656443	8.700694	
16													
17													
18													
19	Channel Cycling A.Yellow												
20													
21	ID	Page 1	1	2	3	34	35	36	37	38	39	40	
22	1	100% MUTANT	7.453269	7.86995	8.056529	10.66235	10.73801	10.95944	11.11844	11.26205	11.38725	11.491	
23	2	100% WILD TYPE	7.567966	8.036055	8.205488	31.78683	33.16724	34.5801	35.344	36.25402	37.17185	37.90588	
24	3	Cutoff Control	7.598884	8.041759	8.241632	31.89624	33.35927	34.74229	35.83607	36.913	37.88652	38.65662	
25	5	Sample 1	7.020793	7.374177	7.537194	14.55286	15.15679	15.61717	16.24475	16.78144	17.33911	18.01442	
26	6	Negative Control	7.079678	7.451567	7.604206	8.598363	8.665364	8.717757	8.75288	8.740495	8.815606	8.868671	

Open analysis tool and Follow the instruction given for analysis

TRUPCR® MTHFR Kit

Instructions for use

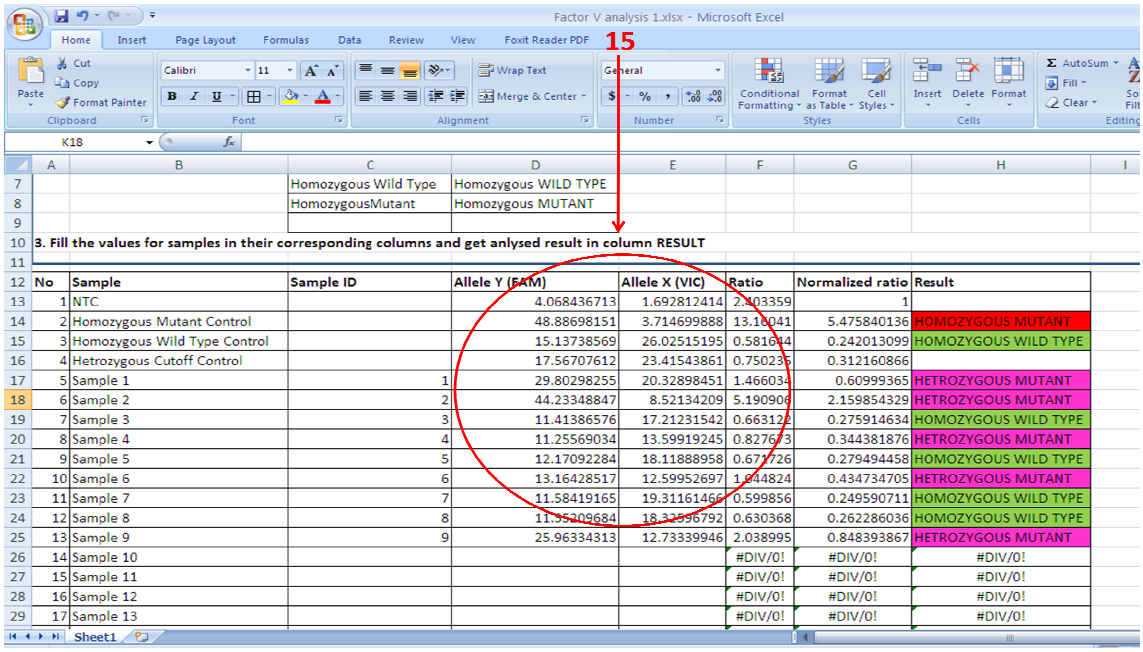
1. After Post Run take the values of Allele X and Allele Y for Homozygous Wild type, Homozygous Mutant, Hetrozygous Cutoff and Non template control.
2. First fill the values for Non template, Hetrozygous Cutoff, Homozygous Wild type and Homozygous Mutant controls and check the Result column, if its matches as given below then proceed for sample analysis

Controls	Result
Homozygous Wild Type	Homozygous WILD TYPE
HomozygousMutant	Homozygous MUTANT

3. Fill the values for samples in their corresponding columns and get anlysed result in column RESULT

No	Sample	Sample ID	Allele X (HEX)	Allele Y (FAM)	Ratio	Normalized ratio	Result
1	NTC				#DIV/0!	#DIV/0!	
2	Homozygous Mutant Control				#DIV/0!	#DIV/0!	#DIV/0!
3	Homozygous Wild Type Control				#DIV/0!	#DIV/0!	#DIV/0!
4	Hetrozygous Cutoff Control				#DIV/0!	#DIV/0!	
5	Sample 1				#DIV/0!	#DIV/0!	#DIV/0!
6	Sample 2				#DIV/0!	#DIV/0!	#DIV/0!
7	Sample 3				#DIV/0!	#DIV/0!	#DIV/0!
8	Sample 4				#DIV/0!	#DIV/0!	#DIV/0!
9	Sample 5				#DIV/0!	#DIV/0!	#DIV/0!
10	Sample 6				#DIV/0!	#DIV/0!	#DIV/0!
11	Sample 7				#DIV/0!	#DIV/0!	#DIV/0!
12	Sample 8				#DIV/0!	#DIV/0!	#DIV/0!
13	Sample 9				#DIV/0!	#DIV/0!	#DIV/0!
14	Sample 10				#DIV/0!	#DIV/0!	#DIV/0!
15	Sample 11				#DIV/0!	#DIV/0!	#DIV/0!

Fill the values of Green and Yellow channel in given column for controls and samples in analysis tool (15), it will automatically give mutation status of sample in Result column



		Homozygous Wild Type	Homozygous WILD TYPE				
		HomozygousMutant	Homozygous MUTANT				
3. Fill the values for samples in their corresponding columns and get anlysed result in column RESULT							
No	Sample	Sample ID	Allele Y (FAM)	Allele X (VIC)	Ratio	Normalized ratio	Result
1	NTC		4.068436713	1.692812414	2.403359	1	
2	Homozygous Mutant Control		48.88698151	3.714699888	13.16041	5.475840136	HOMOZYGOUS MUTANT
3	Homozygous Wild Type Control		15.13738569	26.02515195	0.581614	0.242013099	HOMOZYGOUS WILD TYPE
4	Hetrozygous Cutoff Control		17.56707612	23.41543861	0.750236	0.312160866	
5	Sample 1	1	29.80298255	20.32898451	1.466034	0.60999365	HETROZYGOUS MUTANT
6	Sample 2	2	44.23348847	8.52134209	5.190906	2.159854329	HETROZYGOUS MUTANT
7	Sample 3	3	11.41386576	17.21231542	0.663122	0.275914634	HOMOZYGOUS WILD TYPE
8	Sample 4	4	11.25569034	13.59919245	0.827673	0.344381876	HETROZYGOUS MUTANT
9	Sample 5	5	12.17092284	18.11888958	0.671726	0.279494458	HOMOZYGOUS WILD TYPE
10	Sample 6	6	13.16428517	12.59952697	1.044824	0.434734705	HETROZYGOUS MUTANT
11	Sample 7	7	11.58419165	19.31161466	0.599856	0.249590711	HOMOZYGOUS WILD TYPE
12	Sample 8	8	11.35209684	18.32596792	0.630368	0.262286036	HOMOZYGOUS WILD TYPE
13	Sample 9	9	25.96334313	12.73339946	2.038995	0.848393867	HETROZYGOUS MUTANT
14	Sample 10				#DIV/0!	#DIV/0!	#DIV/0!
15	Sample 11				#DIV/0!	#DIV/0!	#DIV/0!
16	Sample 12				#DIV/0!	#DIV/0!	#DIV/0!
17	Sample 13				#DIV/0!	#DIV/0!	#DIV/0!

ANALYTICAL SENSITIVITY

The analytical sensitivity of the TRUPCR® MTHFR Kit was determined by preparing mixture of Homozygous Wild type samples and Homozygous Mutant samples in different ratio to get range of MTHFR mutation percentage. Five different levels of were tested in triplicates. The analytical sensitivity of kit is determined as ≤ 1% mutant alleles. The result obtained are summarized below

MTHFR Mutation %	Standard Deviation	Coefficient of Variation (%)
0.1	1.23	3.21
1	0.71	2.65
2	0.56	1.95
10	0.28	1.16
50	0.15	0.74

COMPARISON BETWEEN TRUPCR® MTHFR KIT AND SEQUENCING

DNA samples from 35 patients were tested in parallel with the TRUPCR® MTHFR KIT and the gold standard reference technique direct sequencing. Comparisons of results obtained from the 35 interpretable samples are summarized in tables below

		SEQUENCING		
		MTHFR Mutation Positive	MTHFR Wild Type	Total
TRUPCR® MTHFR KIT	MTHFR Mutation Positive	17	0	17
	MTHFR Wild Type	0	18	18
	Total	17	18	35

TROUBLESHOOTING REAL TIME PCR

No.	Observation	Probable causes	Comments
1	Amplification signal in negative control	Cross contamination during handling	Check for contamination of kit's component
2	No amplification signal with positive controls and Sample.	Incorrect PCR mixture	Check whether all components are added.
		Missing control sample during DNA mixing	Be careful when pipetting
		Changing DNA during DNA mixing	Write down sample number on the 1.5 ml micro centrifuge tube and the PCR tube
		Leaving reagents at room temperature for a long time or incorrect storage condition	Please check the storage condition and the expiration date(see the kit label) of the reagents and use a new kit, if necessary
		The PCR conditions do not comply with the protocol	Repeat the PCR with corrected settings

STORAGE AND HANDLING

All the components of TRUPCR MTHFR Qualitative Kits should be stored at -20˚C and stable until the date of expiry stated. The reagents can be aliquot and stored at -20˚C in-order to maintain the stability and sensitivity.

MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED

- Adjustable pipettes with sterile filter or positive displacement tips
- Disposable powder-free gloves
- Sterile bidistilled water
- Sterile 1.5 ml and 2 ml micro centrifuge tubes
- 50 ml conical tubes
- Vortex mixer
- 3B Termi-DNA-Tor or equivalent, in order to remove DNA from working surfaces
- Real time PCR

- Laminar airflow cabinet
- PCR vials (0.2 ml, thin-walled)
- Personal protection equipment (lab coat, gloves, goggles)
- DNA isolation kit

KIT IS COMPATIBLE TO USE WITH FOLLOWING REAL TIME PCR INSTRUMENTS

- Applied Biosystems™ 7500
- StepOne and StepOnePlus
- QuantStudio® 3, 5 and 12
- Rotor-Gene Q
- Bio-Rad CFX96, CFX384
- AriaMx Real-Time PCR
- Roche - LightCycler® 480 -II
- Line gene K real time PCR

GENERAL PRECAUTIONS

The user should always pay attention to the following:

- The product should be delivered on dry ice. Check for presence of dry ice upon arrival.
- Do not use expired products or components.
- Calibrated or verified micropipettes, DNAse, RNAse, pyrogen free micropipette tips with filters, and DNAse, RNAse, pyrogen free micro centrifuge tubes should be used.
- Thaw all components thoroughly at room temperature before starting detection.
- When thawed, mix the components and centrifuge briefly.
- Use disposable gloves, laboratory coats, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- Samples should be considered potentially infectious and handled in a biological cabinet in accordance with appropriate bio safety practices. Infected material and disposable plastic ware that was in contact with infected material must be treated with chlorine-containing solutions.
- Clean and disinfect all sample or reagent spills using a disinfectant, such as 0.5 % sodium hypochlorite or other suitable disinfectant.

- Avoid contact with the skin, eyes and mucosa. If skin, eyes and mucosa contact, immediately flush with water, seek medical attention.
- Material Safety Data Sheets (MSDS) are available on request.
- Use of this product should be limited to personnel trained in the techniques of DNA amplification.
- The laboratory process must be Uni-directional; it should begin in the Extraction Area and then move to the Amplification and Detection Areas. Do not return samples, equipment and reagents to the area in which the previous step was performed.

NOTICE

- The user should always pay attention to the following:
- **Store DNA samples at -20°C until ready for use and keep on ice during use.**
- Avoid microbial contamination of reagents when removing aliquots from reagent tubes. The use of sterile disposable pipette tips is recommended.
- Specimens should be handled as if infectious using safe laboratory procedures. Thoroughly clean and disinfect all work surfaces with 0.5% Sodium Hypochlorite in de-ionized or distilled water.
- This product is to be used by personnel specially trained to perform in vitro diagnostic procedures.
- Although rare, mutations within the highly conserved regions of the viral genome covered by the kit's primers and/or probe may result in under quantitation or failure to detect the presence of the virus in these cases.

REFRENCES

1. Dahlback B et al. Resistance to activated protein C, the FV: Q506 allele, and venous thrombosis. Ann Hematol. 1996; 72:166-176.

2. Arruda, VR, et al. The mutation Ala677. Val in the Methylene Tetrahydro Folate Reductase gene: a risk factor for arterial disease and venous thrombosis. *Thrombosis and Haemostasis* 77(5) (1997).

3. Bagley PJ et al. Proc Natl Acad Sci U S A 1998; 95:13217- 13220.

4. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000 May 1; 151 (9): 862 – 877.

5. Junker R, Nowak-Göötl U, Fobker M. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and other genetic causes of hyperhomocysteinemia in venous thrombosis. J Lab Med. 2001; 25 (7/8): 239 – 253

TRUPCR®

TRUPCR® Molecular Diagnostic Kits

Oncology	**TRUPCR® BCR-ABL Quantitative Kit - M m μ**	*Detection, differentiation and quantitation of BCR-ABL major (M), minor (m) and micro (μ) transcripts. Reporting of Major transcripts ratios on WHO IS.*
	TRUPCR® JAK 2 QT Kit	*Detection and quantitation of Jak2 V617F allele burden on real-time PCR*
	TRUPCR® PML/RARA Quantitative Kit	*Differentiation and quantitation of BCR1, BCR2 and BCR3 transcripts*
	TRUPCR® KRAS Qualitative Kit	*Detection of 22 mutations across codons 12, 13, 59, 61, 117 & 146 of exons 2, 3 & 4*
	TRUPCR® EGFR Mutation Kit	*Detection of 32 different mutations in a single run*
	TRUPCR® AML Panel Kit*	*Qualitative detection of diagnostic markers (AML1-ETO, CBFB MYH11, BCR ABL1 and PML RARA) and prognostic markers (FLT3 ITD/TKD, C KIT and NPM1) of acute myelogenous leukaemia (AML) in peripheral blood samples using real time and conventional PCR system.*
	TRUPCR® ALL Panel Kit*	*Detection and differentiation of fusion genes (E2A/PBX1, TEL/AML1, MLL-AF4, MLL-ENL, MLL AF9 and BCR ABL1) associated with acut lymphoblastic leukaemia.*
* All Markers are also available as individual test.	**TRUPCR® Leukemia Panel Kit***	*Detection of E2A-PBX1, TEL-AML1, MLL-AF4, BCR-ABL1, CBFB MYH11, AML1-ETO, PML-RARA & ABL1 in single panel kit on real-time PCR*
	TRUPCR® MPN Mutation Panel Kit*	*Detection of BCR-ABL1, JAK-2, CALR & MPL in single panel kit on real-time PCR*
Genetics	**TRUPCR® HLA B27 Kit**	*Detection of highest number of HLA B27 allelic subtypes*
Infectious Disease	**TRUPCR® MTB/NTM Nested Kit**	*Detection of Mycobacterium DNA from any sample type on real-time PCR*
	TRUPCR® H1N1 Detection Kit	*Based on CDC certified primers and probes for the detection of type A influenza virus, pandemic influenza A virus and pandemic H1N1 influenza virus*
Drug Resistance	**TRUPCR® Rifampicin Resistant MTB Detection Kit**	*Detection of MTBC & Rifampicin resistance from any sample type*
Coagulation Factor	**TRUPCR® Thrombophilia Panel Kit**	*Detection of 3 Markers: Factor V, Factor II, MTHFR in single panel kit on real-time PCR*
Virology	**TRUPCR® CMV QT Kit**	*Detection and quantitation of Cytomegalovirus on real-time PCR*
	TRUPCR® HSV 1/2 Kit	*Detection of Herpes Simplex virus 1 & 2 on real-time PCR*
Tropical Diseases	**TRUPCR® Dengue/Chikungunya/ Malaria Kit**	*Simultaneous detection of Dengue & Chikungunya and P.falciparum, P.Vivax & Mixed infection on real-time PCR*
Women's Health	**TRUPCR® HPV 16/18 Kit**	*Detection & differentiation of HPV 16 and HPV 18 genotypes on real-time PCR*
	TRUPCR® HPV HR-16/18 Kit	*Detection of 14 High risk HPV genotypes & differentiation of HPV 16 and HPV 18 on real-time PCR*

TRUPCR®

TRUPCR® Molecular Diagnostic Kits

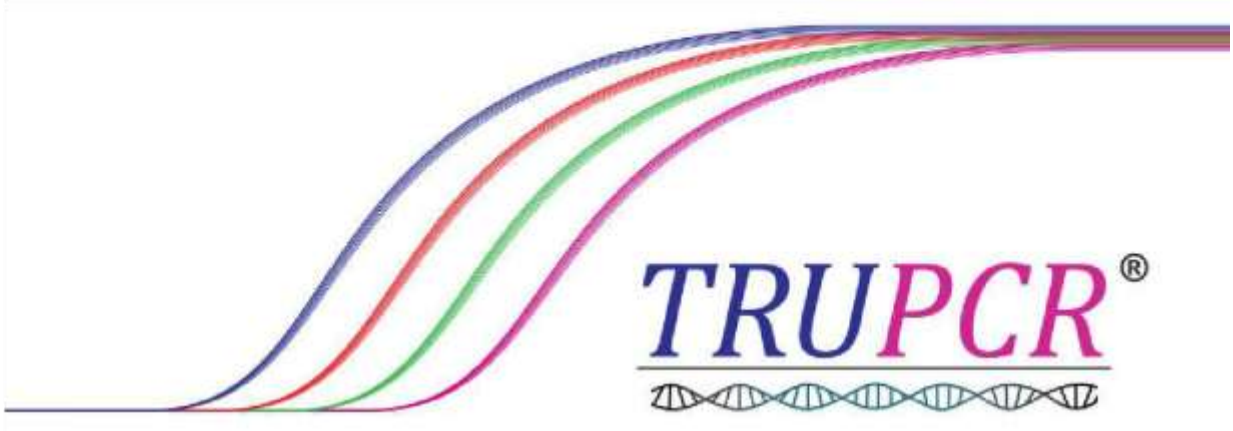
Oncology	**TRUPCR® BCR-ABL Quantitative Kit - M m µ**	*Detection, differentiation and quantitation of BCR-ABL major (M), minor (m) and micro (µ) transcripts. Reporting of Major transcripts ratios on WHO IS.*
	TRUPCR® JAK 2 QT Kit	*Detection and quantitation of Jak2 V617F allele burden on real-time PCR*
	TRUPCR® PML/RARA Quantitative Kit	*Differentiation and quantitation of BCR1, BCR2 and BCR3 transcripts*
	TRUPCR® KRAS Qualitative Kit	*Detection of 22 mutations across codons 12, 13, 59, 61, 117 & 146 of exons 2, 3 & 4*
	TRUPCR® EGFR Mutation Kit	*Detection of 32 different mutations in a single run*
	TRUPCR® AML Panel Kit*	*Qualitative detection of diagnostic markers (AML1-ETO, CBFB MYH11, BCR ABL1 and PML RARA) and prognostic markers (FLT3 ITD/TKD, C KIT and NPM1) of acute myelogenous leukaemia (AML) in peripheral blood samples using real time and conventional PCR system.*
	TRUPCR® ALL Panel Kit*	*Detection and differentiation of fusion genes (E2A/PBX1,TEL/AML1, MLL-AF4, MLL-ENL, MLL AF9 and BCR ABL1) associated with acut lymphoblastic leukaemia.*
	TRUPCR® Leukemia Panel Kit*	*Detection of E2A-PBX1, TEL-AML1, MLL-AF4, BCR-ABL1, CBFB MYH11, AML1-ETO, PML-RARA & ABL1 in single panel kit on real-time PCR*
	TRUPCR® MPN Mutation Panel Kit*	*Detection of BCR-ABL1, JAK-2, CALR & MPL in single panel kit on real-time PCR*
Genetics	**TRUPCR® HLA B27 Kit**	*Detection of highest number of HLA B27 allelic subtypes*
Infectious Disease	**TRUPCR® MTB/NTM Nested Kit**	*Detection of Mycobacterium DNA from any sample type on real-time PCR*
	TRUPCR® H1N1 Detection Kit	*Based on CDC certified primers and probes for the detection of type A influenza virus, pandemic influenza A virus and pandemic H1N1 influenza virus*
Drug Resistance	**TRUPCR® Rifampicin Resistant MTB Detection Kit**	*Detection of MTBC & Rifampicin resistance from any sample type*
Coagulation Factor	**TRUPCR® Thrombophilia Panel Kit**	*Detection of 3 Markers: Factor V, Factor II, MTHFR in single panel kit on real-time PCR*
Virology	**TRUPCR® CMV QT Kit**	*Detection and quantitation of Cytomegalovirus on real-time PCR*
	TRUPCR® HSV 1/2 Kit	*Detection of Herpes Simplex virus 1 & 2 on real-time PCR*
Tropical Diseases	**TRUPCR® Dengue/Chikungunya/ Malaria Kit**	*Simultaneous detection of Dengue & Chikungunya and P.falciparum, P.Vivax & Mixed infection on real-time PCR*
Women's Health	**TRUPCR® HPV 16/18 Kit**	*Detection & differentiation of HPV 16 and HPV 18 genotypes on real-time PCR*
	TRUPCR® HPV HR-16/18 Kit	*Detection of 14 High risk HPV genotypes & differentiation of HPV 16 and HPV 18 on real-time PCR*

* All Markers are also available as individual test.

To know more about complete product range & technical details please visit our website

www.3bblackbio.com

3B BlackBio Biotech India Ltd
7-C, Industrial Area, Govindpura, Bhopal - 462023 (M.P.) INDIA
Email: info@3bblackbio.com Web: www.3bblackbio.com
Phone: +91 755 4076518 ; 4077847 Fax: +91 755 2580438



info@3bblackbio.com

www.3bblackbio.com