

Version 1.2

For Myeloproliferative neoplasms multi-gene panel testing



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# TRUPCR® MPN Panel Kit Version 1.2

Myeloproliferative neoplasms multi-gene panel testing



REF Product No.: 3B1303/3B1304

¥48 tests/ 96 tests

Temperature Limitation

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Manufactured By-

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CONTENT	PAGE NO.
INTENDED USE	3
MYELOPROLIFERATIVE NEOPLASM	3
PRINCIPLE	5
REAGENTS	6
EXTRACTION	7
REVERSE TRANSCRIPTION PCR PROTOCOL	7
REAL TIME PCR PROTOCOL	8
RESULT ANALYSIS FOR FUSION GENES	10
RESULT ANALYSIS FOR MPL	10
RESULT ANALYSIS FOR CALR	11
RESULT ANALYSIS JAK2	11
PERFORMANCE CHARACTERISTIC	12
STORAGE AND HANDLING	13
MATERIAL AND DEVICES REQUIRED BUT NOT PROVIDED	13
GENERAL PRECAUTIONS	14
REFRENCES	14



#### **INTENDED USE**

The **TRUPCR® MPN panel Kit** is intended for the qualitative detection of JAK2-V617F, CALR, MPL W515K/L/A, MPL S505N mutations and BCR ABL1 translocation in Myeloproliferative neoplasms (MPNs) in peripheral blood samples using real time PCR system.

#### **MYELOPROLIFERATIVE NEOPLASM (MPN)**

Myeloproliferative neoplasms (MPN), previously called myeloproliferative disorders (MPD), are a group of diseases that are caused by an overproduction of one or more blood cell types (red cells, white cells or platelets) in the bone marrow. Myeloproliferative neoplasms (MPNs) are associated with dysregulation of tyrosine kinases, leading to abnormal downstream signaling pathways and increased cellular proliferation. Abnormally high numbers of blood cells accumulate in the bone marrow and peripheral blood leading to complications over time. Based on the presence or absence of the Philadelphia chromosome (BCR/ABL1translocation), MPN are broadly grouped into two categories - Philadelphia positive (chronic myeloid leukemia) and Philadelphia negative (polycythemia vera, essential thrombocythemia and myelofibrosis).

CML is characterized by the BCR-ABL1 translocation, t(9;22)(q34;q11.2), which leads to creation of the constitutively active oncogenic BCR-ABL1 fusion tyrosine kinase. This translocation is the most common abnormality in CML.

The Philadelphia chromosome negative MPNs are characterized by mutations in various genes such as JAK2, MPL, CALR, PDGFRA, PDGFRB, FGFR1 and KIT. In addition, multiple chromosome abnormalities have been defined.

#### **BCR ABL1**

The Philadelphia chromosome, corresponding to the BCR ABL1 rearrangement, is the cytogenetic hallmark of chronic myelogenous leukemia (CML) and is frequently found in high-risk acute lymphoblastic leukemia (ALL). In addition, 0.5–3 %of all acute myelogenous leukemia (AML) cases also carries this fusion gene. BCR-ABL-positive acute myeloid leukemia (AML) is a rare subtype of AML that is now included as a provisional entity in the 2016 revised WHO classification of myeloid malignancies. The most common BCR-ABL transcripts (p190 and p210)



are nearly equally distributed. The prognosis of BCR-ABL+ AML seems to depend on the cytogenetic and/or molecular background rather than on BCR-ABL itself.

#### MPL

MPL is located on chromosome 1p34 and encodes for the receptor forthrombopoietin, the key growth and survival factor for megakaryocytes. MPLW515L was first described in 2006 amongst JAK2 V617F-negative PMF patients and is the most frequent MPN-associated MPL mutation, resulting from a G to T transition at nucleotide 1544 on exon 10, causing a tryptophan to leucine substitution at codon 515. Somatic MPL mutations are rare, stem cell derived events involving both myeloid and lymphoid progenitors and limited to MPN patients.

#### **CALR**

Calreticulin (CALR) is a multifunctional protein that acts as a major Ca(2+)-binding protein in the lumen of the cellular endoplasmic reticulum. Calreticulin is encoded by the CALR gene on the chromosome19. Somatic mutations in exon 9 of CALR are the second most prevalent acquired nucleotide changes in Ph-negative myeloproliferative neoplasms (MPNs), except of polycythaemia vera (PV). The two specific mutations are most common, L367fs\*46 (Type 1 mutation) which represents a 52-bp deletion flanked by 7 base pairs of identical sequence and a K385fs\*47(Type 2), which results from a 5-bp insertion, and representing an inverse duplication of the five nucleotides preceding the insertion.

#### **JAK2 V617F**

BCR-ABL1-negative MPN frequently harbor an acquired single nucleotide mutation in JAK2 characterized as c.G1849T; p. Val617Phe (V617F) and it is a gain-of-function mutation that leads to clonal proliferation. The JAK2 V617F is present in 95% to 98% of polycythemia vera (PV), and 50% to 60% of primary myelofibrosis (PMF) and essential thrombocythemia (ET). It has also been described infrequently in other myeloid neoplasms, including chronic myelomonocytic leukemia and myelodysplastic syndrome. Diagnostic criteria for ET, MF, and PV adopted by the World Health Organization (WHO) include identification of a clonal marker, with a specific recommendation to test for the JAK2 V617F mutation in exon 14



#### **PRINCIPLE**

TRUPCR® MPN panel Kit is a Real time assay for qualitative detection of JAK2-V617F, CALR, MPL W515K/L/A, MPL S505N mutations and BCR ABL1 translocation in Myeloproliferative neoplasms (MPNs). In real-time PCR, the analysis is based on fluorescent signal generated from the presence of an oligonucleotide probe specific for target DNA sequence. The probe contains a fluorescent dye molecule on its 5' end and a quencher molecule on its 3' end. When the probe is intact, the proximity of the reporter dye to the quencher dye results in suppression of the reporter fluorescence primarily by Fluorescence resonance energy transfer (FRET).The probe hybridizes with one of the chains of the amplified fragment. During synthesis of a complementary chain, Taq DNA polymerase which possesses 5' - 3' exonuclease activity cleaves the probe. As a result, the fluorescent dye and quencher dye are separated, and the total fluorescence of reaction volume increases in direct proportion to the number of amplicon copies synthesized during PCR.

TRUPCR® MPN Panel kit requires cDNA as template for fusion gene and DNA for other mutation detection. The DNA and RNA both should be extracted from the samples and then RNA should be converted to cDNA using given components in kit.

No	Gene	Variant	Technique
1	BCR-ABL1	e13a2 & e14a2 (p210)	Real Time PCR
1	DCN-ADL1	e1a2 (p190)	Real Time PCR
2	CALD	Type1 (L367fs*46)	Real Time PCR
2	CALR	Type2 (K385fs*47)	Real Time PCR
		W515L	Real Time PCR
3	MPL	W515K	Real Time PCR
3	IVIPL	W515A	Real Time PCR
		S505N	Real Time PCR
4	JAK2	JAK2 V617F	Real Time PCR

List of detectable mutations by TRUPCR MPN Panel kit



#### **REAGENTS**

The Kit contains amplification reagents for performance of 24/48 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.

#### **REAGENTS FOR REVERSE TRANSCRIPTION**

Description	Volume in μL 24 reactions	Volume in μL 48 reactions
RT mix	204 μL	408 μL
Enzyme mix	36 μL	72 μL

#### **REAGENTS FOR PCR**

Reagent	Description	Volume in μL 24 rxn	Volume in μL 48 rxn
Multiplex Master Mix	Mix for Real time PCR	1200 μL x 2	1200 μL x 4
BCR-ABL1 Major Primer Probe Mix (1)	Primer and probe mix for Major BCR-ABL1 detection	120 μL	120 μL x 2
BCR-ABL1 Minor Primer Probe Mix (2)	Primer and probe mix for Minor BCR-ABL1 detection	120 μL	120 μL x 2
ABL1 Primer Probe Mix (3)	Primer and probe mix for ABL1 detection	120 μL	120 μL x 2
MPL-W515L Primer Probe Mix(4)	Primer mix for MPL-W515L detection	120 μL	120 μL x 2
MPL-W515K Primer Probe Mix(5)	Primer mix for MPL-W515K detection	120 μL	120 μL x 2
MPL-W515A Primer Probe Mix(6)	Primer mix for MPL-W515A detection	120 μL	120 μL x 2
MPL-S505N Primer Probe Mix(7)	Primer mix for MPL-S505N detection	120 μL	120 μL x 2
CALR Type 1 Primer Probe Mix(8)	Primer Probe mix for Type 1 mutation detection	120 μL	120 μL x 2
CALR Type 2 Primer Probe Mix(9)	Primer Probe mix for Type 2 mutation detection	120 μL	120 μL x 2
JAK2 V617F Primer Probe mix (10)	Primer mix for JAK2 detection	120 μL	120 μL x 2



Positive control	Positive control A Positive control B JAK2 Mutant Control JAK2 Wild Type Control JAK2 Cut Off Control	50 μL 150 μL 20 μL 20 μL 20 μL	100 μL 300 μL 40 μL 40 μL 40 μL
RNase free Water	RNase free Water	1000 μL	1500 μL

#### **EXTRACTION**

The samples should be shipped at 2 to 8 °C and should be stored at 4°C. To prevent significant degradation of transcripts, samples should be processed within 72 hours of collection, although ideally samples should be processed within 24-36 hours.

RNA and DNA Extraction from EDTA-Blood or Bone marrow can be performed with a recommended procedure using any of the following kit:

Sample Material	Nucleic Acid Isolation Kit	Cat No.
EDTA Blood/Bone Marrow	TRUPCR PANEL Extraction kit	3B1401E

For most sensitive measurement the extracted RNA should immediately be converted in to cDNA and cDNA should then be used in PCR reaction immediately.

The extracted RNA can be store at -80°C for future use.

#### REVERSE TRANSCRIPTION PCR PROTOCOL

#### A. REACTION PREPARATION

Name of the Reagent	Quantity per reaction
RT Mix	8.5 μΙ
Enzyme Mix	1.5 μΙ
Sample RNA*	1 μg
Total reaction volume	20 μΙ

#### NOTE:

1. \*Add up to 10  $\mu$ l of sample RNA (1  $\mu$ g/rxn) and the OD 260/280 of the RNA should be measured spectrophotometrically and should be between 1.7 and 2.0.



#### B. **PROGRAM SET UP**

Define the following setting for Temperature Profile for cDNA Preparation

Step	Temperature, °C	Time	Cycles
1	25	10 min	1
2	47	60 min	1
3	70	05 min	1

#### **REAL TIME PCR PROTOCOL**

#### C. REACTION PREPARATION FOR SAMPLES

NOTE: TRUPCR® MPN Panel kit is a multi-tube format kit. Hence, each sample will be split in separate tubes for separate marker detection.

Prepare 10 tubes for mix preparation and name them 1 to 10 for JAK2 V617F prepare 3 extra reactions for positive controls.

Reagent	Tube 1 Major	Tube 2 Minor	Tube 3 ABL1	Tube 4 MPL W515L	Tube 5 MPL W515K	Tube 6 MPL W515A	Tube 7 MPL S505N	Tube 8 CALR Type 1	Tube 9 CALR Type 2	Tube 10 JAK2 V617F
Multiplex Master Mix	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ	10 μΙ
Major PPM (1)	5 μΙ	-	-	-	-	-	-	-	-	-
Minor PPM (2)	-	5 μΙ	-	-	-	-	-	-	-	-
ABL1 PPM (3)	-	-	5 μΙ	-	-	-	-	-	-	-
MPL W515L PPM (4)	-	-	-	5 μΙ	-	-	-	-	-	-
MPL W515K PPM (5)	-	-	-	-	5 μΙ	-	-	-	-	-
MPL W515A PPM (6)	-	-	-	-	-	5 μΙ	-	-	-	-
MPL S505N PPM (7)	-	-	-	-	-	-	5 μΙ	-	-	-
CALR Type 1 PPM (8)	-	-	-	-	-	-	-	5 μΙ	-	-
CALR Type 2 PPM (9)	-	-	-	-	-	-	-	-	5 μΙ	-
JAK2 V617F PPM (10)	-	-	-	-	-	-	-	-	-	5 μΙ
Total	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ	15μΙ



- a. Transfer 15  $\mu$ l of the above prepared PCR Master Mix in 0.2 ml PCR tubes or plate.
- b. For Tube no. 1, 2 and 3 (BCR-ABL1 and ABL1) add **5 μl** of cDNA.
- c. For Tube no. 4 to 10 (MPL W515L, W515K, W515A, S505N, CALR Type 1, CALR Type 2 and JAK2 V617F) add upto **5 μl** DNA (100ng).
- d. Add Positive Controls 5 μl as follows

Positive control	Tube	Marker
Positive Control A	1, 2, 3	BCR-ABL1 and ABL1
Positive Control B	4,5,6,7,8,9	MPL W515L, W515K, W515A, S505N, CALR Type 1 and
		CALR Type 2
JAK2 Mutant Control	10	JAK2 V617F
JAK2 Wild Type Control	10	JAK2 V617F
JAK2 Cut Off Control	10	JAK2 V617F

#### D. PROGRAM SET UP

As JAK2 is allelic description based assay, post read run is required after the completion of standard real time PCR Run.

#### 1. Real Time PCR:

Define the following setting for Temperature Profile and Dye Acquisition

Step	Temperature, °C	ature, °C Time Dye Acquisition		Cycles
1	94	10 min	-	1
	94	15 sec	-	
2	60	01 min	FAM & VIC/HEX	40

Passive Reference Dye - None

#### 2. Genotyping Assay Run ( Allelic Discrimination): Select only post read run

Step	Temperature, °C	Time	Dye Acquisition	Cycles
1	60	1 min	FAM & VIC/HEX	1

#### 3. CHANNEL SELECTION

Define the following setting for channel selection

Detection	Detector channel	Reporter	Quencher	Gain Setup
All markers /JAK2 Mutant	Green	FAM	None	Auto
Internal Control*/JAK2 Wild Type	Yellow	VIC/Hex	None	Auto

<sup>\*</sup>MPL W515L, MPL W515K, MPL W515A, MPL S505N, CALR Type 1 and CALR Type 2 include endogenous internal control.



## **RESULT ANALYSIS FOR FUSION GENE (BCR ABL1)**

Analyse the result from Standard Real Time PCR Run.

Amplification Signals					
Case ABL1 (Tube 3)		Major BCR-ABL1 (Tube 1)	Minorr BCR-ABL1 (Tube 2)	Interpretation	
1	Present <sup>#</sup>	Present	Absent	Sample is positive for Major BCR- ABL1 translocation	
2	Present <sup>#</sup>	Absent	Present	Sample is positive for Minor BCR- ABL1 translocation	
3	Present <sup>#</sup>	Absent	Absent	Sample is Negative for all fusion genes	
4	Absent	Absent	Absent	PCR inhibition, retest the sample	

<sup>#</sup> To avoid false negativity the ABL1 should be detected on or before 26<sup>th</sup> Cycle.

#### **RESULT ANALYSIS FOR MPL MUTATION**

Analyse the result from Standard Real Time PCR Run.

	Amplification Signals in Hex Channel	Amplification Signals (FAM Channel)				
Coco	MPL W515L (Tube 4)	MPL W515K (Tube 5)	MPL W515A (Tube 6)	MPL S505N (Tube 7)	Interpretation	
1	Present <sup>#</sup>	Present	Absent	Absent	Absent	Sample is positive for MPL W515L mutation
2	Present <sup>#</sup>	Absent	Present	Absent	Absent	Sample is positive for MPL W515K mutation
3	Present <sup>#</sup>	Absent	Absent	Present	Absent	Sample is positive for MPL W515A mutation
4	Present <sup>#</sup>	Absent	Absent	Absent	Present	Sample is positive for MPL S505N mutation
5	Present <sup>#</sup>	Absent	Absent	Absent	Absent	Sample is negative for MPL mutation.
6	Absent	Absent	Absent	Absent	Absent	PCR inhibition or suboptimal amount of DNA: Proceed with a new DNA extraction

<sup>#</sup> To avoid false negativity the Internal Control should be detected on or before 28<sup>th</sup> Cycle.

<sup>#</sup> Any amplification after 34<sup>th</sup> cycle should not be consider positive

<sup>#</sup> Any amplification after 36<sup>th</sup> cycle should not be consider positive



#### **RESULT ANALYSIS FOR CALR MUTATION**

Analyse the result from Standard Real Time PCR Run.

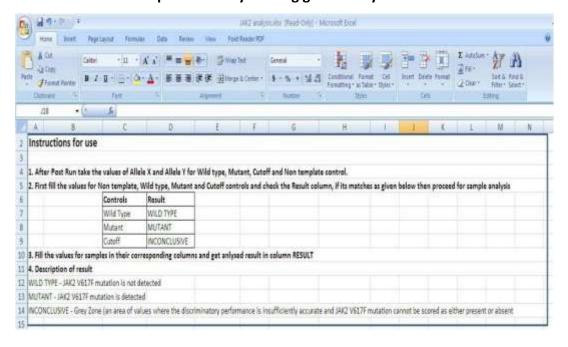
	Amplification	Amplification Signals (FAM		
	Signals in	Channel)		Interpretation
Case	Hex Channel	CALR Type 1 CALR Type 2		interpretation
	(Tube 8 & 9 )	(Tube 8)	(Tube 9)	
1	Present <sup>#</sup>	Present	Absent	Sample is positive for CALR Type 1 mutation
2	Present <sup>#</sup>	Absent	Present	Sample is positive for CALR Type 2 mutation
3	Present <sup>#</sup>	Absent	Absent	Sample is negative for CALR mutation.
1	4 Absent Absent Absent	Absent	PCR inhibition or suboptimal amount of DNA:	
4		Absent	Absent	Proceed with a new DNA extraction

# To avoid false negativity the Internal Control should be detected on or before 28th Cycle.

# Any amplification after 36<sup>th</sup> cycle should not be consider positive

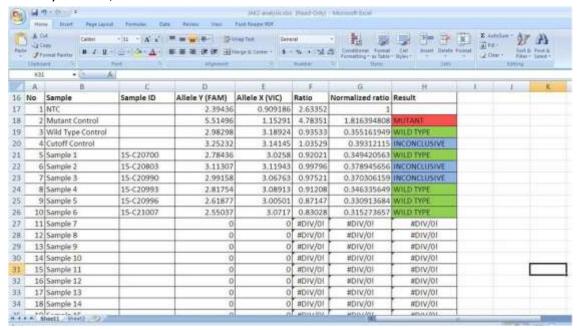
#### **RESULT ANALYSIS FOR JAK2 V617F MUTATION**

- 1. Analyse the result from Genotyping PCR Run (Post Read).
- 2. After completion of run, note down the values of Allele X Rn and Allele Y Rn of each control and sample and analyse using given analysis tools.





Fill the sample ID and value of allele X and allele Y in given column for each sample in analysis tools, and find the result in result column



#### PERFORMANCE CHARACTERISTIC

#### **LIMIT OF DETECTION (LOD):**

The limit of detection (LoD or analytical sensitivity) was determined following CLSI/NCCLS EP17-A2 documents by analyzing dilution series of plasmid containing target genes. For fusion gene-BCR ABL1 the LOD was found to be 10 copies and for MPL, CALR and JAK2 the LOD was found to be equal to 2% mutant allele in background of 98% wild type allele.

#### NOTE:

- 1. The users must be trained and familiar with real time PCR technology prior to the use of this kit.
- 2. Any diagnostic results generated must be interpreted in conjunction with other clinical or laboratory findings.
- 3. It is the user's responsibility to validate system performance for any procedures used in their laboratory which are not covered by the TRUPCR performance studies.
- 4. Attention should be paid to expiration dates printed on the box and labels of all components. Do not use expired components.



#### STORAGE AND HANDLING

All the components of TRUPCR® MPL Panel Kit should be stored at -20°C and stable until the date of expiry stated. The reagents can be aliquoted 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 microcentrifuge tubes
- 50 ml conical tubes
- Vortex mixer
- Heating-block for incubation at 70°C
- Water Bath
- Desktop centrifuge
- Real time PCR
- Laminar airflow cabinet
- PCR vials (0.2 ml, thin-walled)
- 96 100% ethanol
- Personal protection equipment (lab coat, gloves, goggles)

#### KIT IS COMPATIBLE TO USE WITH FOLLOWING REAL TIME PCR INSTRUMENTS

- Applied Biosystems™ 7500
- StepOne and StepOnePlus
- QuantStudio<sup>®</sup> 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:

- Use sterile pipette tips with aerosol barriers and use new tip for every procedure.
- 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.
- Do not use a kit after its expiration date.
- Dispose of all samples and unused reagents in compliance with local authorities' requirements.
- 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 PCR.
- 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.

#### **REFERENCES**

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# **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*  TRUPCR® ALL Panel Kit*	Qualitative detection of diagnostic markers (AML1-ETO, CBFL MYH11, BCR ABL1 and PML RARA) and prognostic markers (FLT ITD/TKD, C KIT and NPM1) of acute myelogenous leukaemia (AML) in peripheral blood samples using real time and conventional PCL system.  Detection and differentiation of fusion genes (E2A/PBX1,TEL/AML1
		MLL-AF4, MLL-ENL, MLL AF9 and BCR ABL1) associated with acu lymphoblastic leukaemia.
* All Markers are also available as	TRUPCR® Leukemia Panel Kit*	Detection of E2A-PBX1, TEL-AML1, MLL-AF4, BCR-ABL1, CBFE MYH11, AML1-ETO, PML-RARA & ABL1 in single panel kit on real-time PCR
individual test.	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

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

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