JAK2 V617F Mutation RT-PCR Detection Kit

Σ 100 Reactions | -20°C

INTENDED USE

JAK2 V617F Mutation Detection Kit is a Real time PCR *in vitro* diagnostic kit for the detection of JAK2 (Janus Kinase 2) V617F allele in genomic DNA extracted from EDTA whole blood against a background of wild type genomic DNA. The mutation observed is a Guanine to Thymidine transversion in position 1849 of the JAK2 gene, which leads to a valine to phenylalanine substitution in position 617 of the protein.

BACKGROUND

The JAK2 gene provides instructions for making a protein that promotes the growth and division of cells. This protein is part of a signaling pathway called the JAK/STAT pathway, which transmits chemical signals from outside the cell to the cell nucleus. Somatic mutations in the JAK2 gene are associated with essential thrombocythemia, a disorder characterized by an increase in number of platelets, the blood cells involved in normal blood clotting. The most common mutation (V617F) replaces the protein building block (amino acid) valine with the amino acid phenylalanine at position 617 in the protein. This particular mutation is found in approximately half of people with essential thrombocythemia.

The V617F JAK2 gene mutation results in the production of a JAK2 protein that is constantly turned on, which in essential thrombocythemia,leads to overproduction of abnormal blood cells called megakaryocytes. Because platelets are formed from megakaryocytes, the over production of megakaryocytes results in an increased number of platelets. Excess platelets can cause abnormal blood clotting, which leads to many signs and symptoms of essential thrombocythemia

PRODUCT DESCRIPTION

JAK2 V617F Mutation Detection Kit is a Real time PCR *in vitro* diagnostic kit for the detection of JAK2 (Janus Kinase 2) V617F allele in genomic DNA extracted from EDTA whole blood against a background of wild type genomic DNA.
Beta Actin is used as the internal control.This kit is intended for "Professional Use only".

ANALYTICAL SPECIFICATION

The specificity of the kit is 100% with 100% sensitivity. The limit of detection of the kit for JAK 2 target Gene is 10 copies per µL

MATERIALS PROVIDED

2X Master Mix (MM) 2 * 625 µL	20X Primer & Probe Mix (PPMx) 125 µL	Nuclease Free Water (NFW) 1000 µL	Positive Test Control (PTC) 100 µL	Instruction for use (1 No)

MATERIALS PROVIDED

KIT COMPONENTS	VOLUME
2X Master Mix (MM)	2*625 µL
20X Primer and Probe Mix (PPMx)	125 µL
Nuclease Free Water (NFW)	1000 µL
Positive Test Control (PTC)	100 µL

TARGET	REPORTER	QUENCHER
JAK 2 Gene	FAM	BHQ1
Beta-actin	TEXAS RED/ROX	BHQ2

*Select the quencher settings as BHQ/None

STORAGE AND HANDLING

- Store all JAK2 Kit kit components at -20 °C.
- Do not repeatedly freeze-thaw reagents as it leads to reduced assay sensitivity. Thaw the reagents only on ice or at 4°C. Recommended Freeze thaw cycle is 5 times.
- Kit components are stable through the end of the expiration date indicated on the box when stored at -20°C. Shelf Life - 12 Months from date of manufacturing

PRECAUTIONS

- This product is recommended to be used by the trained professional under real-time PCR and *in-vitro* diagnostics procedure
- Treat all the specimen/sample as potentially infectious.
- Wear protective disposable powder-free gloves, a laboratory coat and eye protection when handling specimens.
- Store all the collected human samples away from the kit components before use to avoid contamination.
- Handle master mix and template preparation separately and work under biosafety cabinets.
- Use aerosol barrier pipette tips and frequently change the gloves.
- Do not open the reaction tubes/plates post-amplification, to avoid contamination with amplicons.
- Do not smoke, drink or eat in areas where kit reagents and/or human specimens are being used.
- Do not use kit components that have passed their expiration date.

WASTE DISPOSAL

- Dispose all the waste/remains of the reagents used in reaction mixture preparation and expired kit components along with bio-waste as per the lab manual/general bio-waste management instruction
- Dispose the PCR plates with patient samples "sealed" post run to avoid potential infection to the operators and contamination of the lab.

REACTION MIXTURE - 25 µL

Reagents	1 Rxn	25 Rxn	50 Rxn	100 Rxn
2X Master Mix	12.5 µL	312.5 µL	625 µL	1250 µL
20X Primer and Probe mix	1.25 µL	31.25 µL	62.5 µL	125 µL
Nuclease Free Water	5.25 µL	131.25 µL	262.5 µL	1000 µL
Total	19 µL	475 µL	950 µL	1900 µL

Add 6 µL of the test DNA per reaction

TEST PREPARATION/REACTION SET-UP

1. Thaw all components of the kit on ice, mix gently using vortex and spindown the contents for 5 secs and use it immediately.
2. Calculate the number of reactions for each experiment including all controls with one excess reaction volume in the reaction cocktail to accommodate pipetting errors. (eg: number of reaction (n) including controls are 10 add 1 extra reaction during the preparation n+1)
3. Prepare the reaction mix in a 1.5/2 mL tube for the calculated number of samples in Master Mix Preparation room.
4. Spin down the tubes and dispense 19 µL reaction mix in each tube strips or 96 well plate. Before moving to template adding area, add 6 µL of nuclease free water in NTC wells.
5. Carefully add 6 µL samples kept on ice in the designated wells in template addition room. Add 2 µL of PTC in a separate hood and make up the volume by adding 4 uL of nuclease free water. The assay should be run along with positive controls and negative controls.

THERMAL CYCLIC CONDITIONS

STEP	TEMP °C	TIME	DETECTION	CYCLE
Hold	95	2 Min	Off	1
PCR and Detection	95	15 Sec	Off	40
	58	30 Sec	On	

READING TEST RESULTS / DATA ANALYSIS

- NTCs should be negative and should not exhibit fluorescence amplified curves that cross the threshold line.
- If a false positive occurs with one or more of the primer and probe in NTC reactions , it indicates sample contamination.
- In that case, Invalidate the run and repeat the assay with stricter adherence to the procedure guidelines.
- Positive control should produce a positive result with an expected Ct value for each target included in the test.
- If expected positive reactivity is not achieved, invalidate the run and repeat the assay with stricter adherence to procedure guidelines.
- After completion of the run, analyze the data as per the instrument manufacturer instructions.
- Analysis should be performed separately for each target using a manual threshold settings
- In case internal control has not worked for a sample, reperform the test with 2 or more dilutions.
- Positive results for JAK2 should not be used as the sole basis for the treatment.

RESULTS INTERPRETATION

For JAK2	Beta-actin Internal Control	Assay result
Ct < 40	Ct ≤ 40	Positive
Ct = Undetermined or Ct ≥ 40	Ct ≤ 40	Negative
Ct = Undetermined or Ct ≥ 40	Ct ≥ 40 Undetermined	Invalid. Re-purify the nucleic acid from the sample, then repeat the test.

DATA INTERPRETATION

JAK 2 FAM	IC Texas Red/ROX	RESULTS INTERPRETATION
+	+	JAK2 V617F mutation detected
–	+	JAK2 V617F mutation not detected
–	–	Invalid results repeat the DNA extraction and re-run

TROUBLESHOOTING

Positive control showed no amplification

Inappropriate storage of reagents

- Store the reagents at recommended temperature for their optimal performance.
- Avoid freeze-thaw more than the recommended number of times
- Check the expiry of reagents.

Negative controls are positive

Causes - Cross-contamination

- Follow good laboratory practices to avoid contamination issues.
- Use a new batch of reagents and repeat the experiment.

Abnormal plot and/or low ΔRn values in amplification curve

The baseline was set improperly (some samples have CT values lower than the baseline value)

- Switch from manual to automatic baseline, or move the baseline stop value to a lower CT (2 cycles before the amplification curve for the sample crosses the threshold)

An amplification signal is detected in the early cycles

Dilute the sample to increase the CT value