MPL W515L/K Suggested Protocol

MPL W515 L/K Clinical Relevance:

Myeloproliferative disorders (MPD) are a group of haematological malignant diseases characterized by proliferation of one or more hematologic cell lines in the bone marrow. This group includes; Chronic myelogenous leukemia (CML), Polycythaemia Vera (PV), Essential Thrombocythaemia (ET), and Primary Myelofibrosis (PMF), and others. The JAK2 V617F mutation is found in virtually all PV cases and 50-70% of ET and PMF cases. Detection of the JAK2 V617F mutation is an aid in the classification of MPD. A less frequent mutation found in MPD disorders is the MPL W515K or L mutation. The JAK2 V617 and MPL W515K mutations induce constitutive, cytokine-independent activation of the JAK-STAT pathway contributing to uncontrolled cell growth. Researchers have discovered that *MPL*W515L or *MPL*W515K mutations are present in patients with PMF or ET at a frequency of approximately 5% and 1%, respectively, but are not observed in patients with polycythemia vera (PV) or other myeloid disorders. Consequently, detecting MPL515 mutations in JAK2 V617F-negative samples can assist in MPD classification. Studies have also shown that *MPL* mutations may occur concurrently with the *JAK2*V617F mutation, and therefore may provide additional information regarding the characteristics of these MPD cases.

Scope of Procedure:

The following is a recommended procedure for the detection of the MPL W515L and W515K mutations by quantitative PCR. Researchers are advised to utilize this protocol as a guide in the development of their own procedure. This protocol was developed utilizing ancillary reagents and equipment purchased from external vendors. Protocol modifications will be required dependent on the selection of ancillary reagents and equipment utilized in your laboratory.

The following protocol provides instructions for processing samples. The MPL W515L/K primers are designed for allelic discrimination of single base substitutions in the MPL gene. Therefore some cross amplification from non-target DNA is observed. Laboratories are encouraged to develop their own guidelines on the selection and use of standards, controls and data analysis.

CytoGenes Reagents (Required):

Cat #	Item
MPL-WT	MPL-WT Primer Mix
MPL-K	MPL-K Primer Mix
MPL-L	MPL-L Primer Mix

CytoGenes Reagents (Optional):

Cat #	Item	Assay Targets Included	Concentration
MPL-S1	MPL Standard-1	MPL (WT, W515K, W515L)	$5e^5$ copies/ul
MPL-S2	MPL Standard-2	MPL (WT, W515K, W515L)	$5e^4$ copies/ul
MPL-S3	MPL Standard-3	MPL (WT, W515K, W515L)	$5e^3$ copies/ul
MPL-NC	MPL Negative Control	MPL WT	10ng/ul
MPL-PC	MPL Low Positive Control	MPL (WT, W515K, W515L)	6.5% MPL W515L and W515K in normal DNA background

Ancillary Reagents (Recommended):

- iTaqSupermix (BioRad)

Equipment and Supplies (Required):

- Quantitative PCR instrument
- PCR plates, tubes and sealing film.
- Pipettors (range 1-1000ul)
- Aerosol barrier pipette tips.
- Microcentrifuge

Protocol:

1. Isolate DNA from samples utilizing standard procedures utilized by your laboratory.

2. Prepare separate PCR reactions for each of the PCR primer sets to be tested. (Note: for multiple samples, prepare a master mix and scale the volumes appropriately based on the number of samples to be tested).

Reagent	Volume (ul)
iTaqSupermix	10
CytoGenes 25X PCR Primer Mix	0.8
Nuclease Free Water	7.2
Sample	2
Total volume	20

3. Prior to initiating the run, program Q-PCR instrument to detect the probes utilizing the appropriate filter sets. The probes in each of the master mixes are labeled with a FAM reporter and BHQ quencher.

4. Initiate PCR protocol appropriate for a Q-PCR reaction. The following is a recommended protocol based on our instruments and ancillary reagents utilized.

Cycle	Step	Temp (°C)	Time (Min:Sec)	Repeats
1	1	95	3:00	1
2	1	95	:20	40
	2	60	:30	

5. Analyze results.

References:

1. Pardanani AD, Levine RL, Lasho T, Pikman Y, Mesa RA, Wadleigh M, Steensma DP, Elliott MA, Wolanskyj AP, Hogan WJ, McClure RF, Litzow MR, Gilliland DG, Tefferi A. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood. 2006 Nov 15;108(10):3472-6. Epub 2006 Jul 25. PubMed PMID: 16868251.

2. Pikman Y, Lee BH, Mercher T, McDowell E, Ebert BL, Gozo M, Cuker A, Wernig G, Moore S, Galinsky I, DeAngelo DJ, Clark JJ, Lee SJ, Golub TR, Wadleigh M, Gilliland DG, Levine RL. MPLW515L is a novel somatic activating mutation in myelofibrosis with myeloid metaplasia. PLoS Med. 2006 Jul;3(7):e270. PubMed PMID: 16834459; PubMed Central PMCID: PMC1502153.