



# **AMOY Dx ROS1 GENE FUSIONS DETECTION KIT**

Irregularities in chromosomes especially in translocation and the parallel fusion of genes have proved to be an important stage in tumorigenesis. The recognition of gene fusion promises an important step in personalized decisions for cancer treatment. One such biomarker that is looked for in non-small-cell lung cancer (NSCLC) is inaccuracy in the ROS1 gene. In NSCLC patients, the fusion of the ROS1 gene takes place with a portion of another gene. This results in the activation of the ROS1 gene and the outcome is an uncontrolled growth of cells and ultimately cancer. This change in genes is known as ROS1 fusion or ROS1 arrangement (1). An orphan receptor is encoded by the ROS1 gene which is a tyrosine kinase. It belongs to the family of insulin receptors and is related to leukocyte receptor tyrosine kinase and anaplastic lymphoma kinase (ALK) (2).

The fusion of the part of the ROS1 gene containing tyrosine kinase with various other protein partners results in the activation and triggering of survival and growth signaling pathways driving the proliferation of cells (1). In 2007, tyrosine kinase as an active representation of ROS1 arrangement was discovered for the first time, and approximately two percent of the NSCLC patients possess ROS1 fusion (3). The latest clinical research has proved that treatment with Crizotinib offers great benefit to patients having advanced NSCLC bearing ROS1 arrangement (4–7). However, lung cancer patients have a low incidence of ROS1 fusion, because of this, the efficient ROS1 status determination in NSCLC patients is of great importance in patient care.

## **METHODS TO DETECT ROS1 GENE FUSION**

Several methodologies are used for the ROS1 fusion detection. The traditional method used for ROS1 rearrangement detection is by using purported double 'break-apart' probes of fluorescence in situ hybridization (FISH). In this, the two ends of the ROS1 fusion are separated and consequently into the two probes (8–11). Another approach used for detection is to identify the irregular DNA sequence formed in response to the gene rearrangement by utilizing considerably similar 'next-generation' sequencing (NGS). Another opportunity is provided by the mRNA gene fusion following transcription for detection with the technology of polymerase chain reaction (PCR) utilizing multiplex stages having an ability to identify a number of known ROS1 fusion gene transcripts.



## **AmoyDx ROS1 GENE FUSIONS DETECTION KIT — AN RT-PCR ASSAY**

AmoyDx ROS1 Gene Fusions Detection Kit is an RT-PCR assay that is designed to identify fourteen of the most commonly occurring ROS1 rearrangements (12). The kits aim to help clinicians in recognizing the NSCLC patients by identifying the status of ROS1 gene in them so that they can be treated with ROS1 inhibitor therapy (Crizotinib). According to the scientific data, this diagnostic test has been successful in recognition of NSCLC ROS1-positive patients with a specificity of eighty-five to hundred percent and a sensitivity of hundred percent while FISH is used the method of standard reference (13,14).

### **PRINCIPLE AND PROCEDURE**

The procedure is two-step protocol and consist of three step. The first step is the preparation of sample to separate total RNA from sample of FFPE NCSLS. The second step is the reverse transcription of total RNA. In third step, the cDNA generated due to reverse transcription is amplified with the help of PCR by utilizing specific primers and fluorescent probes that are labeled with FAM detect the mutant amplicon while the fluorescent probe that is labeled with HEX detect the reference gene amplicon.

### **WHAT DOES THE KIT CONTAIN?**

The kit contains a ROS1 RT Reaction Mix having primer particularly for reverse transcription for both reference gene RNA and ROS1 RNA into complementary DNA (cDNA). The second component is a ROS1 reaction mix in which primers and probes labeled with FAM specific to ROS1 rearrangement is present in tube 1 to 4 while probe labeled with HEX is present in tube four for identification of reference gene to disclose the existence of inhibitors of PCR or RNA integrity because of which the result could be false negative. The third component is ROS1 positive control (PC) having a ROS1 gene fusion with recombinant gene. The fourth and the final component of the kit is ROS1 enzyme mix in which Taq DNA polymerase is present responsible for PCR amplification. Also the enzyme mix contains uracil-N-glycosylase that prevent the carryover contamination of PCR amplicon.

### **KEY FEATURES**

- ✓ AmoyDx ROS1 Gene Fusions Detection Kit is designed for diagnostic in-vitro use and it should be used by professionals in laboratories having special trainings for PCR techniques.
- ✓ AmoyDx is European conformity marked and results can be obtained with two and half hours on majority of RT-PCR instruments available in market.



- ✓ The kit quantitatively detects 14 ROS1 rearrangements in total human RNA taken out from tumor tissues of NSCLC patients which were formalin-fixed paraffin-embedded (FFPE).
- ✓ The test is a simple procedure in which sample is applied in only one step.
- ✓ The test has a limit of detection of twenty-five copies per micro liter for each ROS1 fusion.
- ✓ Besides, the kit has been tested with two interfering substances; oxyhemoglobin and triglyceride and found that both substances do not interfere with the results.
- ✓ The test kit has high accuracy and sensitivity and it is able to detect even one percent of mutant DNA in context of genomic DNA of wild type.
- ✓ The test kit is manufactured in a dedicated GMP facility having proper systems of quality management to produce a reliable quality finish product.
- ✓ The Kits are available in the form of pre-loaded or in bulk formats and the presence of internal control makes it reliable.

AmoyDx ROS1 Gene Fusions Detection Kit has been approved to be used as a companion diagnostic for a ROS1 inhibitor manufactured by Pfizer Xalkori (crizotinib). A phase II trial was conducted in East Asia in patients who were confirmed to be ROS1-positive through the validation of the AmoyDx ROS1 Gene Fusions Detection Kit. The study demonstrated durable responses in one hundred and twenty-seven patients with ROS1-positive metastatic NSCLC and ALK-negative (7).

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Alercell is proud to distribute the AmoyDx ROS1 GENE FUSIONS DETECTION KIT

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