FusionQuest™ ROS1

A novel, simple-to-use molecular assay for simultaneous detection of multiple fusion gene partners

Introduction

The proto-oncogene c-ros Oncogene 1 (ROS1) encodes a receptor tyrosine kinase of insulin receptor family. Chromosomal rearrangements involving ROS1 produce chimeric oncoproteins that drive a diverse range of cancers. Tyrosine kinase inhibitors (TKIs) have been demonstrated to be therapeutically effective in patients with ROS1 fusion-positive non-small cell lung cancers. With the regulatory approval of TKIs, identifying patients with ROS1 fusions is highly desired. ROS1 fusions occur only in 1~2% of non-small cell lung cancer patients. Among them, CD74-ROS1 is the most common ROS1 fusion (~44%), followed by EZR-ROS1 (16%), SDC4-ROS1 (14%) and SLC34A2-ROS1 (10%)[1]. These known ROS1 fusions can be detected with various methodologies, such as quantitative Polymerase Chain Reaction (qPCR), by analyzing the tumor's nucleic acids.

The FusionQuest™ ROS1 Assay is designed for the qualitative detection of defined gene fusions of ROS1, covering all observed ROS1 fusions in lung cancer with a known breakpoint based on COSMIC database v94. The assay is performed on Quark Bioscience's NextAmp™ Analysis System, which detects fusion targets utilizing qPCR methodology. Below are the key advantages of NextAmp™ when compared to Fluorescence In Situ Hybridization (FISH):

Methodology	FISH	qPCR-based (NextAmp™)
Operational complexity	Moderate	Simple
Interpretation	Subjective	Objective
Identity of fusion partners	No	Yes
Detect intrachromosomal rearrangement	No	Yes
Fusion gene expression	No	Yes
Time to result	2 days	1 day
Cost	$$	$

Key features

- Simultaneous detection of all ROS1 fusion transcripts, 15 fusion partners consisting of a total of 25 known transcripts, in lung cancer (based on COSMIC v94)
- Capable of analyzing challenging clinical FFPE specimens with low RNA input
- Flexibility: Capacity to add additional targets for detection without sacrificing performance and efficiency

Assay details

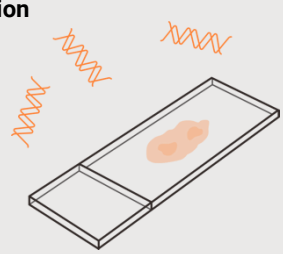
Feature	Specification
Input material	100 ng FFPE extracted RNA
Sample type	FFPE specimen
Target number	15 ROS1 fusion partners
Throughput	1 - 6 samples per run
Run time	Approximately 2 hours
Dynamic range	10 - 10^6 copies
Limit of detection (LoD)	10 copies
Specificity	100%
Linearity	0.998 (0.995 - 1.000)
Reproducibility	0.978 (0.946 – 0.995)

FusionQuest™ ROS1 workflow

Analysis of all known ROS1 fusion targets with 3 simple steps.

Step1
Sample Preparation

RNA extraction
cDNA synthesis
Pre-amplification



Step2
qPCR Reaction

One-step qPCR
run on-a-chip

Step3
Data Analysis

Comprehensive
data analysis with
actionable result

Technology behind qPCR-based NextAmp™ Analysis System

The FusionQuest™ ROS1 is performed on the NextAmp™ Analysis System, which consists of a proprietary thermocycler Q Station™ and PanelChip® with thousands of nanowells to detect multiple biomarkers. Primers of 25 known ROS1 fusion transcripts were designed around the fusion exon points and pre-loaded onto PanelChip®. After simple sample preparation performed on FFPE specimens, subsequent amplification and analysis are carried out on NextAmp™ with results provided in less than 2 hours.

FusionQuest™ ROS1 panel

The FusionQuest™ ROS1 Panel includes 15 fusion partners and 2 internal control genes.

ROS1 fusion partners on FusionQuest™ ROS1 panel are listed below:

ROS1 fusions (25)	CD74-ROS1	CD74 exon 6; ROS1 exon 32
		CD74 exon 6; ROS1 exon34
		CD74 exon 6; ROS1 exon35
	EZR-ROS1	EZR exon 10; ROS1 exon 34
	SDC4-ROS1	SDC4 exon 2; ROS1 exon 32
		SDC4 exon 2; ROS1 exon 34
		SDC4 exon 4; ROS1 exon 32
		SDC4 exon 4; ROS1 exon 34
	SLC34A2-ROS1	SLC34A2 exon 4; ROS1 exon 32
		SLC34A2 exon 4; ROS1 exon 34
		SLC34A2 exon 13; ROS1 exon 32
		SLC34A2 exon 13; ROS1 exon 34
	TPM3-ROS1	TPM3 exon 2; ROS1 exon 36
		TPM3 exon 8; ROS1 exon 35
	ZCCHC8-ROS1	ZCCHC8 exon 2; ROS1 exon 36
	GOPC/FIG-ROS1	GOPC exon 4; ROS1 exon 36
		GOPC exon 8; ROS1 exon 35
	LRIG3-ROS1	LRIG3 exon 16; ROS1 exon 35
	CCDC6-ROS1	CCDC6 exon 5; ROS1 exon 35
	HLA-A-ROS1	HLA-A exon 9; ROS1 exon 34
	MYO5A-ROS1	MYO5A exon 23; ROS1 exon 35
	PPFIBP1-ROS1	PPFIBP1 exon 9; ROS1 exon 35
	ERC1-ROS1	ERC1 exon 12; ROS1 exon 36
	PWWP2A-ROS1	PWWP2A exon 1; ROS1 exon 36
	CLIP1-ROS1	CLIP1 exon 20; ROS1 exon 36

Internal control genes

RNA housekeeping gene 1	ACTB
RNA housekeeping gene 2	GADPH

Reference

1. Drilon et al. (2021). ROS1- dependent cancers — biology, diagnostics and therapeutics. *Nat Rev Clin Oncol. 18(1):35-55*

For more information, please visit www.quarkbiosciences.com

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