

biotechne® / Asuragen®

# *ESR1* Sales Playbook

# Playbook Introduction

## What it *ISN'T*

This Playbook is NOT designed to be an exhaustive, all-encompassing guide on how to sell our QuantideX® qPCR *ESR1* exoMutation Kit\*. This tool assumes you have already qualified your customer and have verified they have sufficient testing volumes, access to necessary instrumentation, appropriate personnel and resources, etc.

## What it *IS*

It is a tool to help you ask more detailed and technical questions to better profile your customers, as well as help you overcome objections you are likely to hear from various users and influencers. In doing so, this will increase our rates of success and stave off our competition.

### Reinforcing Our Value Proposition

The Quantidex qPCR *ESR1* exoMutation Kit is the first multi-analyte liquid biopsy assay tailored for detecting *ESR1* mutations. Designed to provide highly sensitive detection of 11 of the most prevalent *ESR1* variants in plasma, the kit offers a novel approach to achieving more meaningful breast cancer insights.

Use exosomes to achieve unparalleled sensitivity for detection of *ESR1* mutations using conventional qPCR equipment, enabling sample-to-answer testing within a single laboratory shift.

### Competitors:

- **Qiagen:** dPCR *ESR1* Mutation Kit (not released)
- **APIS:** dPCR *ESR1* Mutations Kit (not sold in US)
- **Bio-Rad:** ddPLEX *ESR1* Mutation Kit
- **Illumina:** Next Generation Sequencing

Bringing High-Sensitivity *ESR1* Mutation Testing to qPCR

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# Best-in-Class Against the Competition

Equal to or Better Than the Competition

## Quantitative vs. Qualitative... does it matter?

Based on Clinical trials, no. There is no lower limit of detection. The most important feature is to detect *ESR1* mutations as soon as possible. Quantitation is not necessary.

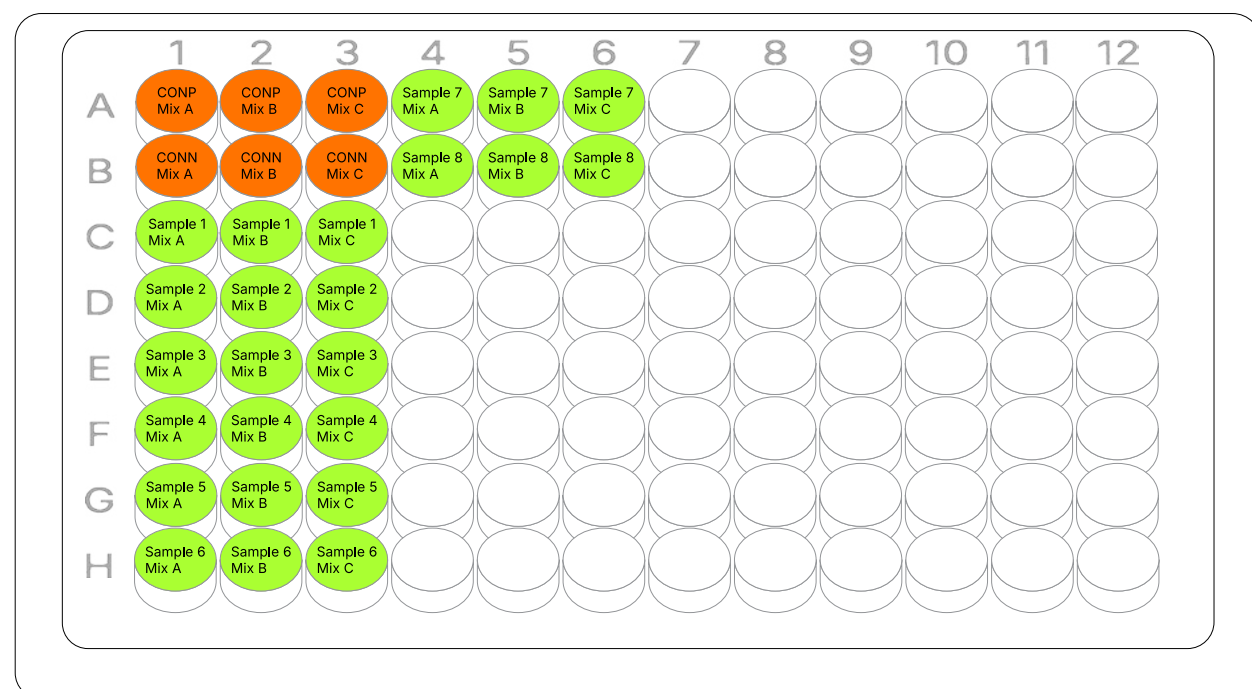
## Key Product Requirements

	Bio-Techne Asuragen	Qiagen	APIS	Bio-Rad	Next Generation Sequencing  <i>(i.e. Illumina)</i>
	QuantideX® qPCR <i>ESR1</i> exoMutation Kit	dPCR <i>ESR1</i> Mutations Kit <i>Not Released</i>	APIS <i>ESR1</i> Mutations Kit <i>Not Sold in US</i>	Bio-Rad ddPLEX <i>ESR1</i> Mutation Kit	
<b>Coverage</b>	11 <i>ESR1</i> mutations Contained in 3 mixes	11 <i>ESR1</i> mutations	11 <i>ESR1</i> mutations Contained in 6 mixes	7 <i>ESR1</i> mutations 2-tube Solution (mut & wt)	Complete <i>ESR1</i> Gene
<b>Assay Sensitivity</b>	<0.03-0.08% VAF (~5 copies/mL)	TBD	LOD (%MAF) from 1.0%–0.1% Note: only 1 mutation at 0.1%. Most at 0.4%	From 0.01%–0.025% VAF, Only D538G at 0.01% VAF	Panels range from 0.25–0.35% VAF. Guardant Health claims 1% VAF with 5 ng input and 0.2% VAF with 30 ng input.
<b>Analyte</b>	Cell-free DNA and Exosomal RNA	Cell-free DNA	Cell-free DNA	FFPE and Cell-free DNA	Cell-free DNA and RNA
<b>Controls Provided</b>	Positive, Negative and Internal Controls	TBD	Positive, Negative and Reference Controls	Positive, Negative and Internal Controls	Positive, Negative and Internal Controls
<b>Time-to-Result / Hands-on-Time</b> <i>Estimated for 8 Samples</i>	5.3 hours from Blood Collection Tube (BCT) to Analysis	TBD	Sample-to-answer in one day (<8 hours)	Sample-to-answer in one day (<8 hours)	7–14 days from Sample to Answer
<b>All-Inclusive Kit</b>	Yes, Reverse Transcriptase (RT), PreAmp, qPCR and ExoLution Plus Isolation Kit	TBD	Yes, complete kit. Not on Biocartis Idylla platform. Just distributed by Biocartis.	Not all-inclusive, additional reagents needed.	No, sequencing reagents sold separately
<b>Results Reporting</b> <i>Push-Button Software</i>	Yes, automated results	TBD	No, manual calculations	Yes, automated results	Yes, automated results
<b>Strengths</b>	<ul style="list-style-type: none"> <li>11 <i>ESR1</i> mutations detected across 3 mixes – based on SERENA-1 trial</li> <li>Assay Sensitivity: 0.03-0.08% MAF</li> <li>Analyte is exoRNA &amp; cfDNA, boosting assay sensitivity</li> <li>Start with as little as 2 mL of Plasma</li> </ul>	<ul style="list-style-type: none"> <li>QIAcuity Dx Digital PCR System is 510(k) exempt in the US and IVDR-certified for diagnostic use in EU</li> </ul>	<ul style="list-style-type: none"> <li>11 <i>ESR1</i> mutations detected</li> <li>Sold by BioCartis, but not on BioCartis platform</li> <li>Kit contains Positive, Negative and Reference (Internal) controls</li> </ul>	<ul style="list-style-type: none"> <li>Assay Sensitivity: 0.01–0.025% MAF</li> <li>Positive, Negative &amp; Internal Controls provided</li> <li>Sample to answer in 1 day</li> </ul>	<ul style="list-style-type: none"> <li>Look at 300–500 genes per panel</li> <li>Look at the entire <i>ESR1</i> gene</li> <li>Detect SNVs, CNV &amp; Fusions in a single seq run</li> </ul>
<b>Weaknesses</b>	<ul style="list-style-type: none"> <li>ExoLution Plus cfDNA and exoRNA Isolation kit included with the <i>ESR1</i> exoMutation Kit.</li> <li>The ExoLution Plus kit is a column based manual kit limiting throughput to 6-12 samples at one time</li> </ul>	TBD	<ul style="list-style-type: none"> <li><i>ESR1</i> mutations are split between 6 mixes</li> <li>Assay Sensitivity depends on specific mutations, ranging from 0.08-1.0%</li> <li>Twice the number of wells (60) for 8 samples compared to Asuragen (30 wells).</li> </ul>	<ul style="list-style-type: none"> <li>Limited to 7 <i>ESR1</i> mutations detected</li> <li>Manual or auto drop generator instrument / reagents required</li> </ul>	<ul style="list-style-type: none"> <li>Sensitivity is 0.25–0.35% VAF</li> <li>Cost per sample significantly higher than qPCR. May have to batch to spread out costs</li> <li>7-14 day TAT</li> </ul>
<b>Regulatory Status</b>	RUO	TBD	RUO	RUO	RUO

# Comparison of Plate Configuration

## QuantideX qPCR *ESR1* Kit

- 3 Reaction Mixes covering 11 *ESR1* mutations
- Each sample must be run in all 3 mixes, Mix A, Mix B, and Mix C
- Both CONP and CONN are run in each of the 3 mixes
- 6 Samples + 2 Controls = 24 reactions – recommended minimum
- 8 Samples + 2 Controls = 30 reactions
- Maximum number of reactions that can be run on a plate is 30 + 2 Controls



The kit is verified for use on the Thermo Fisher Scientific ABI Fast Dx 7500, Quant Studio 5, and QuantStudio™ 7 instruments

## Bio-Rad *ESR1* Mutation Kit

- Two-tube assay
  - 60 reactions of the mutant assay and
  - 60 reactions of the wild-type assay
- The kit can accommodate up to 42 samples per run in “singlet” (1 mutant and 1 wild type) utilizing a 96-well plate with all required controls
- The non-template control (NTC), Negative and Positive Control are run in duplicate for both Mutant and Wild Type

**Table 12. Example plate map for testing controls in duplicate and 18 samples in singlet.**

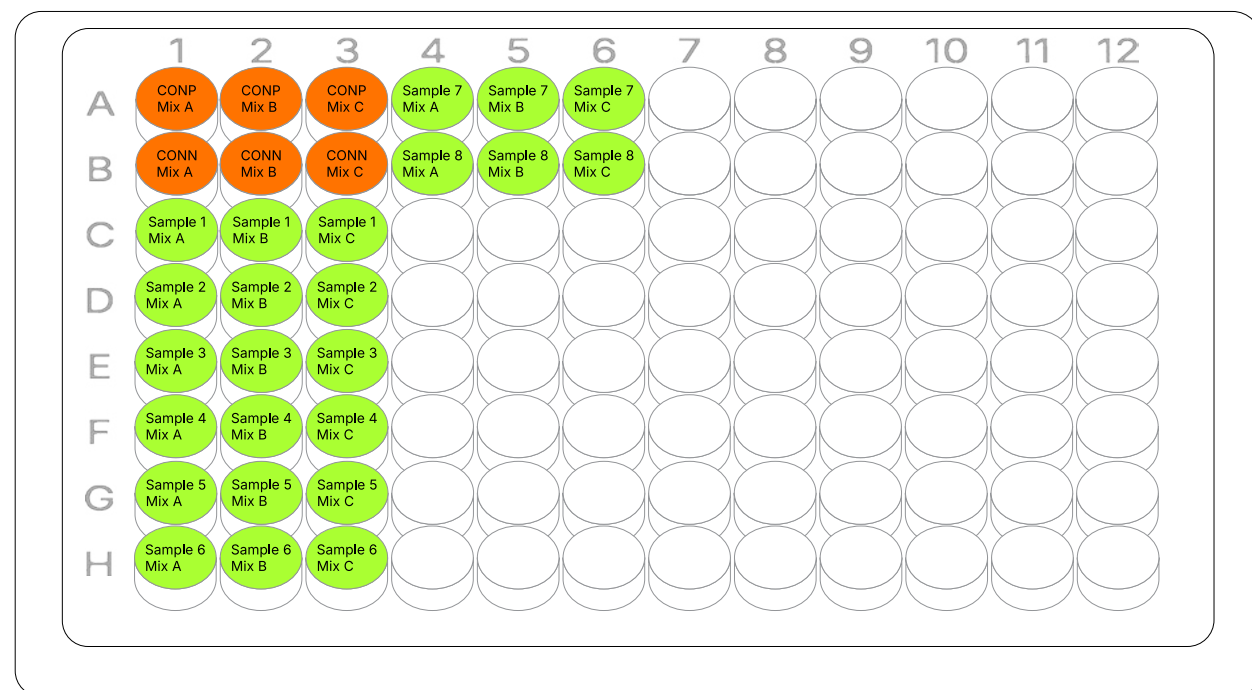
	Mutant	Wild Type				
	1	2	3	4	5	6
A	NTC	NTC	Sample 3	Sample 3	Sample 11	Sample 11
B	NTC	NTC	Sample 4	Sample 4	Sample 12	Sample 12
C	Negative Control	Negative Control	Sample 5	Sample 5	Sample 13	Sample 13
D	Negative Control	Negative Control	Sample 6	Sample 6	Sample 14	Sample 14
E	Positive Control	Positive Control	Sample 7	Sample 7	Sample 15	Sample 15
F	Positive Control	Positive Control	Sample 8	Sample 8	Sample 16	Sample 16
G	Sample 1	Sample 1	Sample 9	Sample 9	Sample 17	Sample 17
H	Sample 2	Sample 2	Sample 10	Sample 10	Sample 18	Sample 18

The kit is for use with Bio-Rad QX600 Droplet Digital PCR (ddPCR) System

# Comparison of Plate Configuration

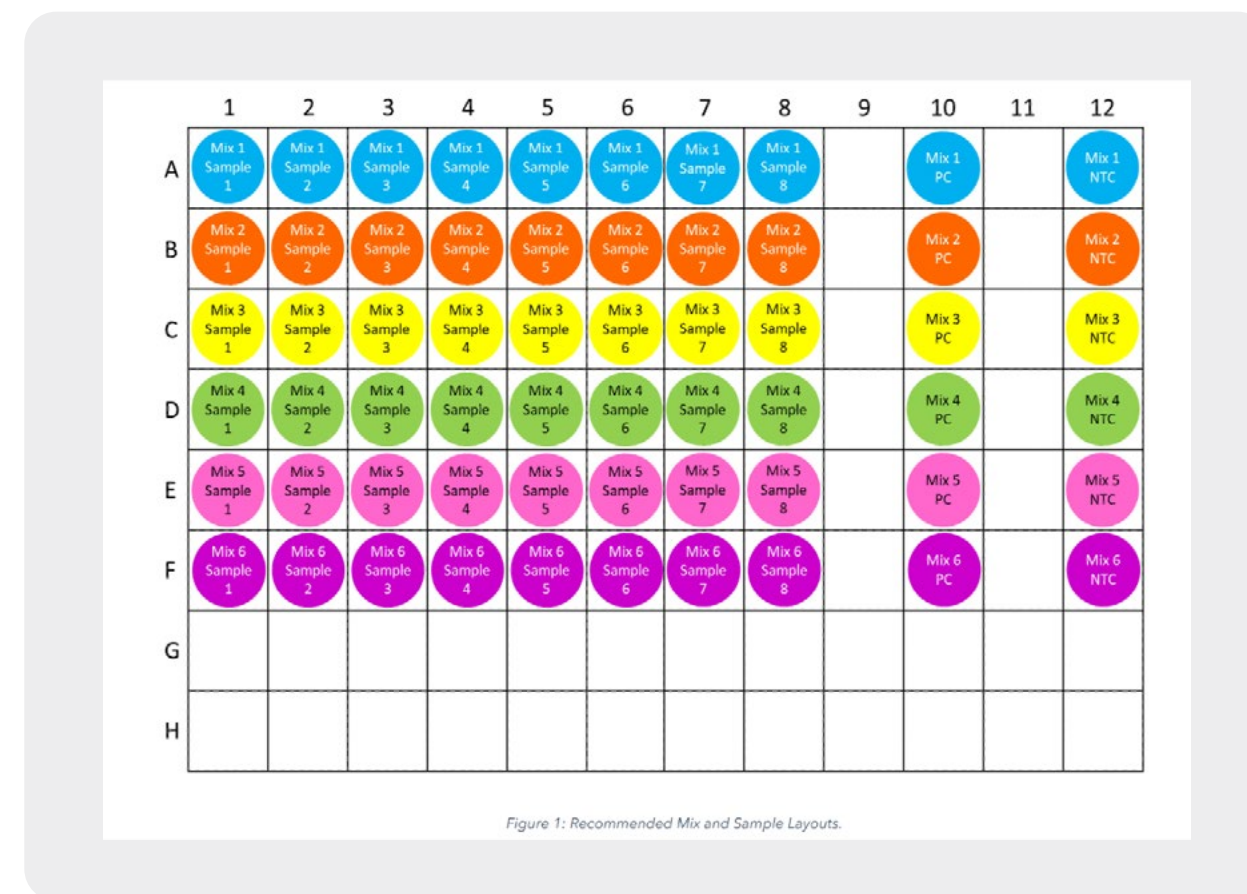
## QuantideX qPCR *ESR1* Kit

- 3 Reaction Mixes covering 11 *ESR1* mutations
- Each sample must be run in all 3 mixes, Mix A, Mix B, and Mix C
- Both CONP and CONN are run in each of the 3 mixes
- 6 Samples + 2 Controls = 24 reactions – recommended minimum
- 8 Samples + 2 Controls = 30 reactions
- Maximum number of samples that can be run on a plate is 30 + 2 Controls



## APIS *ESR1* Mutations Kit

- 6 Reaction Mixes covering 11 *ESR1* mutations
- Each sample must be run in all 6 mixes
- Both PC and NTC are run in each of the 6 mixes
- 8 Samples = 60 reactions – maximum recommended for a plate



Real-Time PCR instrument (calibrated for FAM™ and HEX™ dyes)

# Complete QuantideX<sup>®</sup> qPCR *ESR1* Workflow in a Single Day

## Instruments Supported with Verification Data:

- ABI 7500 Fast Dx
- QuantStudio 5 Dx
- QuantStudio 7 Pro Dx

- Sample Preparation – 40 min
- ExoLution Plus Isolation – 60 min
- *ESR1* mutation assay (RT, PreAmp & qPCR) – 215 min (~3.6 hours)
- Total time ~5.3 hours

- No measurements from ExoLution Plus to RT.
- PreAmp master mix added onto RT products decreases sample handling.
- Internal controls, contained in Mix A, Mix B, & Mix C, confirm if sufficient starting material was added to the reaction.
- Workflow has multiple stopping points so the protocol can be started on one day and completed the next

	Sample Preparation	ExoLution Plus	Reverse Transcription	PreAmp PCR	qPCR	Data Analysis	Total
<b>Hands-On Time</b>	10 min	30 min	10 min	5 min	10 min	2 min	67 min
<b>Instrument Time</b>	30 min	30 min	90 min	25 min	75 min	1 min	251 min
	10 ml blood yields ~4 mL plasma	Transfer 2-4 mL plasma	Transfer entire isolate to RT step. No quantification	Add 7.5 µL of PreAmp master mix into RT rxn well	Dilute PreAmp 1:40 and use 2 µL in qPCR. Total vol is 10 µL.		

# Opening Statements & Key Points

## Opening Statements

- Are you familiar with the biomarker *ESR1* and how monitoring *ESR1* mutations can impact metastatic breast cancer?
  - **If Not**, we have been following two clinical trials that focus on monitoring *ESR1* looking for the early detection of *ESR1* mutations in mBreast Cancer, indicating resistance to first-line therapy. Both studies show the benefit of detecting *ESR1* mutations early and switching from an Aromatase Inhibitor to a new SERD. SERD stands for selective estrogen receptor degrader. SERDs bind to estrogen receptors (ERs) in tumor cells, causing the cells to break down the receptor.
  - **If Yes**, they are familiar with monitoring *ESR1* mutations, I would go into our kit. We have developed an assay that detects 11 *ESR1* mutations (content based on SERENA-1) that detects the mutations down to 0.03-0.08% VAF (variant allele frequency) on a qPCR instrument in less than 6 hours.

# 5 Key Talking Points We Need to Drive Home

- QuantideX qPCR *ESR1* exoMutation Kit covers the 11 most common mutations (associated with treatment resistance).
- The 11 *ESR1* mutations covered are the same as those studied in the SERENA-1 Clinical Trial.
- What is unique about our kit is the input material (analytes). Kit utilizes both cell-free DNA and exosomal RNA.
- Unlike cell-free DNA, which only secretes 2 copies of DNA when the cell dies, exosomes are actively released from all living cells, secreting tens of thousands of exosomes a day.
- Because we utilize both cfDNA and exoRNA as the input material, the assay sensitivity is 0.03-0.08% VAF (for all 11 mutations) from just 2 mL of plasma. Our level of sensitivity is equivalent to digital PCR.

# Product Snapshot

## QuantideX® qPCR ESR1 exoMutation Kit\*

Category	Description & Target Specifications
<b>Coverage</b>	<p>Detects 11 <i>ESR1</i> mutations located in ligand binding domain split into 3 mixes</p> <p>Mix A: D538G, S463P</p> <p>Mix B: Y537X (S,N,C,D) , E380Q</p> <p>Mix C: L536X (H,P,R), V422del</p> <p>Note: "X" denotes multiple mutations at that codon</p>
<b>Size</b>	50 Reactions
<b>Analyte</b>	cfDNA + exoRNA co-isolated with ExoLution Plus Kit
<b>Sample</b>	2 – 4 mL Plasma (double-spun + filtered) K <sub>2</sub> EDTA (purple top) & PAXgene ccfDNA at launch
<b>Controls</b>	Positive Control, Negative Control & Internal Control
<b>Freeze / Thaws</b>	6
<b>Recommended min # of Samples</b>	6 samples + 2 controls = 8 reactions for each of the 3 mixes (A,B & C)
<b>Assay Sensitivity</b>	0.03-0.08% VAF (Variant Allele Frequency) - ~5 copies/mL
<b>Platform</b>	ABI 7500 Fast (Dx), QuantStudio 5 Dx, and QuantStudio 7 Pro Dx
<b>Regulatory</b>	RUO
<b>Workflow</b>	<b>RT:</b> 10 min hands-on time <b>PreAmp:</b> 5 min hands-on time 25 min instrument time <b>qPCR:</b> 10 min hands-on time 75 min instrument time <b>Total Time:</b> ~3.6 Hours
<b>Data Analysis</b>	<b>Qualitative Mutation Detection</b> via Provided Software

# Product Snapshot

## ExoLution™ Plus cfDNA & exoRNA Isolation Kit\*

ExoLution Plus cfDNA & exoRNA Isolation Kit	
<b>Description</b>	<p>Column based method (using a tabletop centrifuge) to isolate exosomal RNA (exoRNA) and cfDNA</p> <p>No ultra centrifugation steps required</p> <p>Included in the purchase of QuantideX qPCR <i>ESR1</i> exoMutation Kit</p>
<b>Analyte Isolated</b>	Exosomal RNA isolated from exosomes and cfDNA from plasma
<b>Workflow</b>	<b>Blood Collection Tube:</b> 5 min hands-on time 25 min instrument time <b>ExoLution Plus:</b> 30 min hands-on time 30 min instrument time
<b>Plasma Input Amount</b>	2 – 4 mL
<b>Storage</b>	Room Temperature
<b>Kit Size</b>	50 Purifications
<b>Kit Contents</b>	All spin columns and plastics, including 50 mL conicals, included

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