AmpliSeq Focus Panel – Preparation and Shipping Guidelines

The quality of the *AmpliSeq Focus Panel* result depends on the following **critical pre-analytical steps** in order to obtain high-quality nucleic acids. This includes fixation, microtome section preparation, specimen preparation and shipping.

1. Fixation:

Fixation of the specimen with only **10% neutral-buffered formalin,** for a period of 24–72 hours, allows preservation of nucleic acids. Other fixatives may lead to false negative results and are not accepted (e.g. Bouin, B5, AZF, Holland's or strong acid decalcification procedures). Under fixation or over fixation can lead to suboptimal results.

2. Microtome Section Preparation:

Ribonucleases (RNases), which is present ubiquitously (eg. normal human skin), can destroy nucleic acids. Careful preparation and clean material are necessary to avoid cross contamination and RNases. Disposable material and smooth tip tweezers cleaned with an ANTI-RNase product (eg. RNase Away™) are encouraged.

1) Wear gloves and change them frequently, especially between specimens
2) Change the microtome blade for each case
3) Use clean instruments to transfer the sections (i.e. sterile needle to transfer the scrolls into clean 1.5 ml Eppendorf tubes)
4) Use hot plate or a clean water bath (for unstained slides)
5) All material (tube, slides) must be adequately labelled (two patient identifiers)

3. Specimen Preparation:

Specimen type	*Requirements*
Scrolls FFPE*	**≥ 10% of tumor cell content (TCC)** in the whole cut surface is required. TCC is the number of viable nucleated tumor cells in proportion to the total number of cells. 1) **10 scrolls** at 5-µm (minimum 5 scrolls) in a **labelled** 1.5 ml Eppendorf tube **2) 1 H&E slide**
Unstained Slides FFPE	Required for microdissection when **TCC <10%** 1) **6 unstained labelled slides** at 5-µm thickness**,** uncharged and unbaked 2) **1 H&E slide**, pointed for tumor area if tumor content < 10%

**FFPE: Formalin-fixed paraffin-embedded. When the tumor cell content (TCC) ≥ 10%, scrolls are preferred.*

Revised & approved by Dr. Gomez 23-10-26

AmpliSeq Focus Panel – Test Information

Indications for ordering

This test allows the detection of genomic abnormalities with a diagnosis, prognosis or predictive role in diverse solid tumours (e.g. lung, lower GI, melanoma).

Test Methodology

The *AmpliSeq Focus Panel* is a targeted next generation sequencing assay for analysis of hotspot regions in 52 genes with known relevance to solid tumours. The panel enables the simultaneous analysis of genes associated with several most common cancers (e.g. lung, colon). The DNA and RNA analysis can detect multiple alterations including: (i) single nucleotide variants (SNVs), (ii) small insertion / deletions (indels), (iii) copy number gains and (iv) gene fusions in RNA samples. See below for gene list. For more information on the hotspot regions tested, please contact the laboratory.

Gene List

DNA (hotspot variations and *amplifications)				
AKT1	*EGFR**	*FGFR4**	*JAK3*	*MYCN**
*ALK**	*ERBB2**	*GNA11*	*KIT**	*NRAS*
*AR**	*ERBB3*	*GNAQ*	*KRAS**	*PDGFRA**
*BRAF**	*ERBB4*	*HRAS*	*MAP2K1*	*PIK3CA**
*CCND1**	*ESR1*	*IDH1*	*MAP2K2*	*RAF1*
*CDK4**	*FGFR1**	*IDH2*	*MET**	*RET*
*CDK6**	*FGFR2**	*JAK1*	*MTOR*	*ROS1*
CTNNB1	*FGFR3**	*JAK2*	*MYC**	*SMO*
DDR2				

RNA (fusions)				
ABL1	*EGFR*	*ETV5*	*NTRK1*	*PPARG*
ALK	*ERBB2*	*FGFR1*	*NTRK2*	*RAF1*
AKT3	*ERG*	*FGFR2*	*NTRK3*	*RET*
AXL	*ETV1*	*FGFR3*	*PDGFRA*	*ROS1*
BRAF	*ETV4*	*MET*		

Revised & approved by Dr. Gomez 23-10-26

Test Interpretation

Only variants with a recognized clinical significance, diagnostic, prognostic or predictive are reported (Tier I and II; PMID: 27993330). Additional results are available upon request.

Note: This test does not detect large deletions.

Type of specimens accepted

- Tumour cell content ≥ 10% is required. This information is mandatory to assess the validity of the test.

- Specimen (cytology or histology), frozen or formalin-fixed paraffin-embedded (FFPE) are accepted.

- For FFPE: only SCROLLS or UNSTAINED SLIDES are accepted.

- Unstained slides: a minimum of 5 sections of 5 µm thickness non-baked and uncharged, with 1 H&E slide (tumour area should be outlined with permanent marker if macrodissection is needed).

The full procedure is detailed in the document Preparation and Shipping Guidelines.

Limitations

Results must be interpreted in the **context of clinical, radiological and histological findings.** If results obtained do not match other clinical or laboratory findings, or if you have novel relevant information, please contact the laboratory as soon as possible for updated interpretation.

Results may be compromised if the recommended procedures (tissue fixation and preparation) have not been followed.

A negative (wild type) result does not fully rule out the presence of an alteration but may be linked with limits of detection of this assay (i.e. insufficient % of tumour cell content or poor fixation).

Rare polymorphisms may be present that could lead to false-negative or false-positive results.

Turnaround time: 10 working days