Extraction:

Before starting the extraction:

- ✓ A pathologist must analyse each sample to verify and define the tumour area.
- ✓ The percentage of **tumour cells** should be at least **20%** per sample in order to obtain significant results.

Deparaffination:

Paraffin Slides

1. Immerse the glass slides in **Xylene for 5'**.

2. Immerse the glass slides in **Ethanol 96% for 5'.**

3. Immerse the glass slides in **fresh Ethanol 96% for 5'**.
Withdraw the cover glass and scrape the slice with a scalpel.
Collect the sample on a 1.5ml tube.

4. Centrifuge 5' at max speed.
Discard the supernatant.

5. Centrifuge 2' at max speed.
Discard the supernatant.
Allow the ethanol to air-dry.

Paraffin Rolls

1. Discard as much paraffin as possible from the block before preparing the slices.

2. Take the paraffin rolls into a 1.5 ml tube.

3. Add **500 µl of mineral oil**. Make sure the oil covers the sample completely.

4. Incubate 2' at 95ºC and 550 rpm.

5. Centrifuge 2' at 8000 rpm.

6. Carefully aspirate the oil **avoiding to touch the sample**.

7. Repeat **steps 3-6**.

8. Add the **lysis buffer** making sure it completely covers the sample.

Extraction:

Continue with the extraction with the **QiAmp DNA FFPE Tissue Kit, Qiagen**.
Follow the manufacturer instructions.
Elution volume: 50 µl.

Amplification:

Before starting the amplification:

- ✓ The amount of genetic material added to each amplification tube should be **150 ng** in a max volume of 10 µl.
- ✓ It is recommended to dilute the extracted DNA to **30 ng/µl to add 5 µl to each amplification tube**.
- ✓ If DNA concentration is lower than 15 ng/µl the extraction should be repeated.
- ✓ It is recommended the use of conventional thermocycler or adjust the temperature ramp to the assays needs (see point 7.3 on the user's manual).
- ✓ **Keep the amplification tubes at 4ºC at all times**.

1. Thaw just the number of amplification tubes according to the number of samples to analyze.

The tubes must be thawed at 4ºC

BRAF : **Mix1** (white tube) + **Mix2** (green tube).

BRAF·MEK1·AKT1: **Mix1** (White tube) + **Mix2** (Green tube) + **Mix3** (Red tube).

↓

2. If possible, briefly centrifuge the amplification tubes.

↓

3. Add **5-10 µl of extracted DNA** to each amplification tube.

↓

4. Place the amplification tubes in the thermocycler only when **the block has reached 95ºC**.

↓

5. The amplification product must be **kept at 4ºC and visualized within 5 days**.

PCR program:

1 cycle	95ºC	15'
40 cycles	94ºC	60''
	66ºC	60''
1 cycle	72ºC	10'
1 cycle	4ºC	∞