

Sample Handling Guideline for OTS-Monitor

【A】 Extracted DNA

■ DNA Solution Requirements

- ▷ Solvent: TE buffer or equivalent
- ▷ DNA amount: 5-10 ng per mutation
- ▷ Total DNA: Ensure that the total DNA quantity is sufficient for the number of target mutations
- ▷ DNA concentration: ≥ 0.5 ng/ μ L
- ▷ Volume: ≥ 20 μ L
- ▷ Provide detailed information on the measurement of DNA concentration (Instrument/Kit/Measurement date)

■ Notes

- ▷ Avoid cross-contamination between samples

■ Shipping Conditions

- ▷ Keep $\leq -20^{\circ}\text{C}$ throughout transport

■ Recommended Kits

- ▷ QIAGEN: QIAamp DNA Mini Kit (#51304)
- ▷ NIPPON GENE: ISOSPIN Tissue DNA Kit (#316-08891)



DNA amount ≥ 10 ng
per mutation

【B】 Plasma

■ Plasma Separation Procedure

- ▷ Collect 8 – 10 mL whole blood
- ▷ Immediately after blood collection, centrifuge at $1,800 \times g$ for 20 min at room temperature using a swinging-bucket rotor.
- ▷ Transfer 4 – 5 mL plasma to a new 15 mL tube
- ▷ Centrifuge again at $1,800 \times g$ for 20 min at room temperature, using a swing rotor
- ▷ Carefully transfer the supernatant (plasma) to a new cryovial without disturbing the pellet. Immediately store at -80°C

■ Notes

- ▷ Avoid cross-contamination with blood cells to ensure accurate ctDNA measurement

■ Shipping Conditions

- ▷ Keep $\leq -20^{\circ}\text{C}$ throughout transport

■ Recommended Product

- ▷ Simpot, Cryovial Silicone O-Ring Seal and Internal Threads, 5 ml Tubes Round Bottom (T301-5)



Simpot Cryoval 5ml

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【C】 Whole Blood

■Collection

▷Use Streck Cell-free DNA BCT®

▷Volume: 8 – 10 mL

*If above blood tube is unavailable, separate plasma immediately (refer to section 【B】)

■Shipping Conditions

▷Ship at room temperature (not refrigerated)

▷Must arrive within 5 days of sample collection

▷Ensure tubes are securely protected from breakage

■Recommended Product

▷Streck, Cell-free DNA Blood Collection Tubes (STM-230570)



Streck, Cell-free DNA BCT

◆OTS-Monitor supports the use of plasma DNA or DNA derived from frozen tissue or FFPE as the template for dPCR.

【D】 Frozen Tissue

Depending on the purpose of the analysis, DNA derived from frozen tissue may be used for OTS-Monitor in addition to plasma DNA.

■Requirements

▷umor tissue: 5 – 15 mm³ (6 – 17 mg)

▷Tumor cell content: ≥ 30%

■Shipping Conditions

▷Keep ≤ –20°C throughout transportation

▷Samples will not be returned

■Recommended Product

▷Thermo Fisher Scientific, Nunc™ Biobanking and Cell Culture Cryogenic Tubes (#377267)



Thermo Fisher Scientific
Cryotube 1.8ml

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【E】 FFPE Biopsy Samples

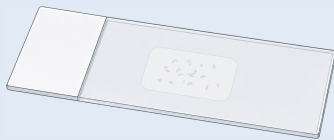
■ Recommended

▷ FFPE block storage period: Less than 1 years (at contact us)

■ Requirements

▷ When unstained slides are available:

1. Unstained slides ($8\mu\text{m}$): 3 slides
 - A) Each slide: $\geq 5\text{-}6$ biopsy tissues ($\sim 2\text{-}3\text{ mm}^2$ each)



× 3 slides

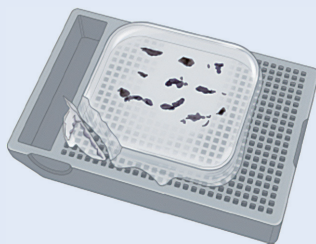
B) If tumor volume is limited:

Increase numbers of slides until total tissue volume reaches 50 mm^3

2. H&E stained slides: 1 slides (from the final section of the same block, with specimen numbers)

▷ When unstained slides are NOT available:

1. FFPE blocks: 1–2 blocks (representative blocks)



2. H&E stained slide: 1 slide (from the final section of the same block, with specimen numbers)
3. A copy of pathology report

【F】 FFPE Surgical Samples

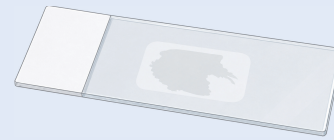
■ Recommended

▷ FFPE block storage period: Less than 5 years (at contact us)

■ Requirements

▷ When unstained slides are available:

1. Unstained slides ($8\mu\text{m}$): 2 slides
 - A) Tumor area per slide: $\geq 25\text{ mm}^2$ (after trimming)



× 2 slides

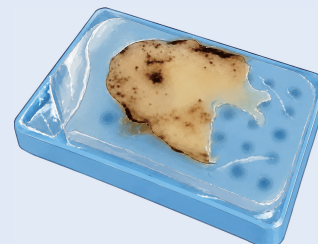
B) If tumor area $< 25\text{ mm}^2$:

Increase numbers of slides until total tissue volume reaches 50 mm^3

2. H&E stained slides: 1 slides (from the final section of the same block, with specimen numbers)

▷ When unstained slides are NOT available:

1. FFPE blocks: 1–2 blocks (representative blocks)



2. H&E stained slide: 1 slide (from the final section of the same block, with specimen numbers)
3. A copy of pathology report

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General Notes for [E] [F]

■ Basic Requirements

- ▷ H&E slides will not be returned
- ▷ Label slides with:
 - Sample ID
 - Serial number of FFPE block
 - * Do NOT include personal identifiers
- ▷ FFPE blocks will be returned within 1–2 months
- ▷ Preparation and shipping costs are the responsibility of the customer
- ▷ Shipping temperature: Room temperature

■ Slide Preparation Conditions

- ▷ Fixation: 10% neutral buffered formalin
- ▷ Bone samples: EDTA decalcification only
- ▷ Tumor content: $\geq 30\%$ (trimming allowed)
- ▷ Slide mounting: up to 2 sections per slide allowed
 - * Multiple sections must be indicated in the submission form
- ▷ Avoid heat stretching: store at room temperature

■ Contamination Prevention

- ▷ Use a new microtome blade
- ▷ Use new gloves
- ▷ Replace water bath water