

Guideline for Collecting and Packing Clinical Samples

For Immune Cell Monitoring with Epiontis ID Technology

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Precision for Medicine**

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General Information

This guideline outlines the requirements for collecting, packing and shipping clinical samples to Epiontis GmbH (part of Precision for Medicine). For the purpose of this guideline, clinical samples are defined as human derived materials (e.g. non-infectious or potentially infectious) including blood, PBMC, tissue and genomic DNA.

Quality Management

Epiontis GmbH (part of Precision for Medicine) is accredited by the German Accreditation Body (“Deutsche Akkreditierungsstelle”, DAkkS). Personnel qualification, equipment and facility maintenance as well as validation are performed according to the international standard DIN EN ISO/IEC 17025 and good clinical practice (GCP).

Input Requirements

Table 1 lists the standard inputs for the parallel measurement of up to four epigenetic assays. Certain combinations of epigenetic assays might result in slightly increased sample requirements. For materials not listed a pilot study is recommended with around 5 separate donors as e.g. tissue type, disease stage, age, fixation or storage can influence the DNA yield.

Generally, sample input requirements for Epiontis ID technology should be discussed directly with customer service representatives for each study.

Sample Material	Sample amount for epigenetic analysis of 1-4 cell types	Recommended total amount for potential repeat analysis	Absolute Quantification applicable
Whole blood (frozen):			
✓ EDTA	75 µl	250 µl	+
✓ Citrate	75 µl	250 µl	+
✓ PAXgene	300 µl	1 ml	-
✓ Tempus	300 µl	1 ml	-
Cells purified from blood (frozen):			
✓ PBMC	0.5 mio cells	1.5 mio cells	-
✓ Enriched/sorted cells, culture	Discuss for each study		-
Fresh frozen tissue (frozen):			
✓ Colon (tumor)	200 µg	600 µg	-
✓ Salivary Gland, Tonsil (non-tumor)	1 mg	2 mg	-
✓ Skin (non-tumor)	3 mg	6 mg	-
✓ Other tissues	Pilot study (~5 samples)		-
FFPE tissue:			
✓ Colon, ovarian, kidney, lung tissue	1 cut (at least 100 mm ² with 10 µm thickness)	3-5 cuts	-
✓ Other tissue	Pilot study (ca. 5 samples)		-
DNA (frozen):			
✓ Genomic DNA	2.5 µg	7.5 µg	-

Table 1: Recommended sample amounts for the measurement of up to 4 epigenetic assays. The input recommendations should be doubled for the parallel measurement of 5-8 assays, tripled for 9-12 assays, quadrupled for 13-16 assays, quintupled for 17-20 assays and sextupled for > 20 assays.

Sample Collection

All sample containers should be labelled only with a barcode and/ or sample ID. Identifiable donor information should not be submitted.

Whole Blood (e.g. EDTA, Citrate, PAXgene, Tempus)

- Collect samples in standard blood collection tubes; gently invert the tube a few times to mix the blood with the anticoagulant.
- Recommendation for whole blood: BD Vacutainer Plus 4 ml lavender/EDTA with Hemogard part number 368861 (Europe) or 367844 (US, other countries).
- Recommendation for RNA stabilized whole blood: PAXgene Blood RNA tubes (BD part number 762165).
- After collection, blood samples can be kept at 4°C for up to 24 hours before freezing at or below -20°C.
- Samples should be shipped on dry ice.

PBMC and purified/sorted cells

- PBMC or purified cells should be provided in standard 1 ml containers (e.g. Eppendorf) as pellets or alternatively re-suspended in PBS buffer (max. volume 75 µl).
- For fluorescence-activated cell sorting (FACS) use external antibodies if possible. If internal staining is needed, the eBioScience Foxp3 staining buffer set is recommended (Cat. No. 5523).
- Cell samples should be stored frozen (if possible, do not supplement DMSO) at or below -20°C and shipped on dry ice.

Fresh Frozen Tissue

- Fresh frozen tissue can be provided in Tissue-Tek® for storage; tissue sample without any added reagents is preferred.
- Tissue samples should be stored frozen without paper, toothpicks etc. at or below -20°C and shipped on dry ice.

Formalin Fixed Paraffin Embedded Tissue Slices (FFPE)

- It is recommended to place single FFPE cuts derived from the same donor in individual Eppendorf tubes (e.g. 3 cuts per sample = 3 tubes, 10 samples with each 3 cuts = 30 tubes; preferred are 0.2 ml tubes).
- Embedded tissue samples should be stored at or below 4°C and shipped with cool pads or on dry ice.

Purified Genomic DNA

- Genomic DNA samples should be provided with a DNA concentration of at least 50 ng/µl and an 260/280 ratio close to 1.8 (in 1.5 ml Eppendorf tubes).

- For preparation of genomic DNA from cells, tissue or whole blood the "DNeasy Blood & Tissue Kit" from Qiagen (Cat. No. 69504) is recommended.
- For genomic DNA derived from enriched cell populations input requirements are lower (less material required) and should be discussed for each study.
- DNA samples should be stored at or below 4°C (for long-term storage at or below -20°C) and can be shipped at room temperature, with cool pads or on dry ice.

Shipment of Samples

Sample Name Clarification

Sample ID, additional sample information, and packaging information are required to be submitted along with samples using the shipment information form. Please ensure that the sample IDs on the tubes and on the shipment information form are consistent. Please, do not provide any sample information that allows donor identification.

General Packaging Requirements

All shipments must comply with all applicable laws governing packing, marking and labeling.

Please ensure that the dry ice load/cool pads will be sufficient to keep samples frozen/cooled during the entire shipment and that the samples are surrounded by cooling material on all sides. Use proper protection for your shipment to prevent sample tubes from breaking.

Address and Package Labelling Information

To avoid any customs delays over the weekend, please ship samples on a Monday or Tuesday. For frozen samples, World Courier or MARKEN are recommended shipping agencies.

If applicable, specify samples in the cover/consignment letter as follows:

- biological samples of human origin,
- non-toxic, non-hazardous, non-infectious,
- for research purpose only,
- commercial value: 1 US \$ / 1 Euro

Collected and packaged specimens should be sent to:

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shipment@epiontis.com

Please contact us prior to shipment to ensure that all details can be clarified and also provide the tracking number of your shipment after package pick up.

The progress of the shipment will be continuously monitored by customer service representatives. The integrity of sample containers and material will be checked upon receipt and sample arrival will be confirmed by E-mail.