

Guideline for Collecting and Packing Clinical Samples

For Epiontis' Epigenetic qPCR Immunomonitoring Service

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General Information

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

Quality Management

Epiontis is accredited by the German Accreditation Body (DAkkS). Personnel qualification, validation, facility and equipment maintenance are performed according to the ISO norm ISO/EN 17025 and the guideline for good laboratory practice (GLP).

Input Requirements

Table 1 provides a guideline for required amounts for the parallel measurement of up to four epigenetic assays. Certain combinations of epigenetic assays might result in slightly increased sample requirements. Reversely the measurement of less than four assays in parallel may need less sample material. Therefore, sample input requirements should be discussed directly with the Epiontis customer service representative for each study.

If materials should be used for which no information is given, please contact the Epiontis customer service. For unlisted sample material Epiontis recommends a pilot study with around 5 separate donors as e.g. tissue type, disease stage, age, fixation or storage can influence the DNA yield.

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, breast, ovarian 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 parallel measurement of up to 4 epigenetic assays. The input recommendations should be doubled for the parallel measurement of 5-8 assays and tripled for 9-12 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 suitable blood collection tubes according to the manufacturer's instructions; 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 Eppendorf tubes 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 (no DMSO) at or below -20°C and shipped on dry ice.

Fresh Frozen Tissue

- Fresh frozen tissue should be provided in 1.5 ml Eppendorf tubes. Tissue-Tek® may be used for storage; tissue sample without any added reagents is preferred.
- Tissue samples should be stored frozen at or below -20°C and shipped on dry ice.

Formalin Fixed Paraffin Embedded Tissue Slices (FFPE)

- Single FFPE cuts derived from the same donor should be placed 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 inputs requirements are

reduced 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 Epiontis 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 local, state and federal 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

Please direct questions regarding sample collection, packaging and/or shipping of samples to shipment@epiontis.com and contact us prior to shipment to ensure that all details can be clarified. In addition, please contact us after package pick up to provide the tracking number of your shipment.

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

It is recommended to 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

Epiontis will monitor the progress of shipment, check the integrity of sample containers and material upon receipt and will confirm sample arrival to the customer.