

Specifications for Biospecimen Qualification Assays

1. Fluid Biospecimens (serum, plasma, urine, CSF)

Test	Scope	Min. input quantity	Readout
sCD40L measurement	Assess the time human serum or EDTA plasma samples have been exposed to room temperature	50µl	<ol style="list-style-type: none"> sCD40L concentration (pg/µl) and assessment of the integrity of serum Fitness for the purpose of proteomic or metabolomic analyses
Creatinine and cystatin C measurement	Normalise urine samples in terms of protein content	150µl	Combinatorial creatinine and cystatin C categorisation of samples
Serum fingerprinting*	Certify the common ancestry of serum biospecimens in terms of "parent" blood samples	100µl	Serological signature
Haemoglobin measurement	Assess the presence of significant amounts of haemoglobin in serum, plasma, CSF, urine	200µl	<ol style="list-style-type: none"> Assessment of haemolysis in a biological fluid Fitness for the purpose of proteomic or metabolomic analyses
CRP measurement	Assess the inflammatory status of serum or plasma samples	100µl	CRP concentration and categorisation of serum or plasma samples
Calprotectin measurement	Assess the inflammatory status of stool samples	40-120mg	Calprotectin concentration and categorisation of stool samples

*Coming soon

LacaScore	Assess the pre-centrifugation delay of EDTA plasma samples	220µl	<ol style="list-style-type: none"> 1. Ascorbate/lactate ratio 2. Fitness for the purpose of metabolomic analyses
IL-8 measurement	Assess the pre-centrifugation delay of serum and citrated plasma samples	110µl	<ol style="list-style-type: none"> 1. Target cytokine concentration 2. Fitness for the purpose of proteomic analyses
IL-16 measurement	Assess the pre-centrifugation delay of EDTA plasma samples	110µl	<ol style="list-style-type: none"> 1. Target cytokine concentration 2. Fitness for the purpose of proteomic analyses, cfDNA, ccfRNA analyses
Fibrinogen measurement	Assess the coagulation efficiency of serum samples and to differentiate serum from plasma samples	10µl	Fibrinogen concentration

2. Nucleic Acids

Test	Scope	Min. input quantity	Readout
DNA quantification by spectrophotometry	Measure the concentration and the purity of DNA extracted from blood, cells, tissue	3µl	Total DNA concentration (ng/µl) and purity (A260/A280)
DNA quantification by spectrofluorometry	Measure the concentration of double-stranded DNA extracted from any material, including low cellularity materials	2µl	<ol style="list-style-type: none"> 1. Double stranded DNA concentration (ng/µl) 2. Prerequisite for NGS applications
RNA quantification by spectrophotometry	Measure the concentration and the purity of RNA extracted from blood, cells, tissue	3µl	RNA concentration (ng/µl) and purity (A260/A280)

RNA quantification by spectrofluorometry	Measure the concentration of RNA extracted from low cellularity materials	2 μ l	RNA concentration (ng/ μ l) for samples of very low concentration
Human DNA quantification (qPCR)	Measure the concentration of DNA of human origin	4 μ l	Human DNA concentration
Bacterial DNA quantification (qPCR)	Measure the concentration of DNA of bacterial origin	2 μ l	Bacterial DNA concentration
DNA cross-linking assessment by multiplex PCR	Assess the degree of cross-linking of human DNA extracted from FFPE tissue samples	100ng	<ol style="list-style-type: none"> 1. Assessment of the degree of DNA cross-linking 2. Fitness for the purpose of WGA and CGH array analyses
DNA fingerprinting	Certify the common ancestry of human biospecimens, in case of doubt	100ng	Assessment of donor identity between samples
Long range PCR	Evaluate the amplifiability of human DNA extracted from blood, cells, fresh tissue	180ng	<ol style="list-style-type: none"> 1. Max. DNA amplicon size (up to 15kb) 2. DNA integrity
RNA integrity number (RIN) and DV200 measurement	Assess the integrity of ribosomal RNA extracted from blood, cells, tissue	1 μ l, 25ng/ μ l min. concentration	<ol style="list-style-type: none"> 1. RIN 2. Fitness for the purpose of gene expression microarray analyses
Size range PCR	Assess the integrity of human RNA transcripts and the maximum amplicon target size that can be analyzed	1 μ g	<ol style="list-style-type: none"> 1. Max. RNA transcript amplicon size (up to 942bp) 2. Fitness for the purpose of qRT-PCR gene expression analyses
DNA integrity number (DIN) measurement	Assess the integrity of DNA extracted from blood, cells, tissue	1 μ l, 5ng/ μ l min. concentration	DIN

Test	Scope	Min. input quantity	Readout
RT-qPCR for miRNA detection	Verify that miRNAs have been co-extracted in human RNA extracts from blood, cells, tissue, fluids	25ng	<ol style="list-style-type: none"> 1. Assessment of the presence of miRNAs 2. Fitness for the purpose of miRNA analyses
SPUD assay	Verify the absence of PCR inhibitors in DNA extracted from any source of material	15µl	<ol style="list-style-type: none"> 1. Assessment of the presence of PCR inhibitors 2. Fitness for the purpose of molecular biology analyses
mRNA quality index	Assess the integrity of human messenger RNA extracted from blood, cells, tissue	80ng, 6.67ng/µl min. concentration	<ol style="list-style-type: none"> 1. Quantitative index indicating the integrity of mRNA 2. Fitness for the purpose of qRT-PCR gene expression analyses
Illumina FFPE QC assay	Assess the integrity of FFPE extracted DNA by qPCR	2ng	<ol style="list-style-type: none"> 1. Quantitative ΔCt index 2. Fitness for the purpose of NGS
cfDNA size measurement	Verify the size of extracted cfDNA	1µl	Assessment of non-contamination by WBC DNA
WGA index	Assess the degree to which whole genome amplification can be achieved in DNA extracted from blood, cells, tissue	100ng	<ol style="list-style-type: none"> 1. Quantitative index indicating DNA integrity and whole genome amplifiability 2. Fitness for the purpose of CGH microarray analyses

3. Cells

Test	Scope	Min. input quantity	Readout
Cell viability and apoptosis	Measure cell viability and early apoptosis	1-3x10 ⁵ cells	Viability and apoptosis %
Cell enumeration	Measure cell numbers	10 ⁴ /ml	Cell number/ml
Purity of cell subpopulations	Measure the purity of sorted lymphocytes, monocytes, granulocytes or other subpopulations	1-10x10 ⁵ cells	% purity of a sorted cell subpopulation
PBMC functional assays (ELISPOT)	Measure the functionality of lymphocytes	1.5x10 ⁶ cells (assay dependent)	Number of spots from Elispot analyser
PBMC preanalytical score (IL-8, EDEM3)	Assess the pre-centrifugation delay of PBMCs from EDTA-anticoagulated blood	100ng total RNA	1.Quantitative ΔCt index 2.Fitness for the purpose of gene expression analyses, or functional analyses
Adipocyte functional assays	Test functionality of mature adipocyte cells by measuring production of adipocyte-derived proteins (Adiponectin, Leptin)	320 μl culture supernatant for both assays	Adiponectin and Leptin concentrations
Induced pluripotent stem cells (iPSC) characterisation by immunofluorescence (IF)	iPSC pluripotency characterization by IF using canonical cell surface markers (SSEA-4, TRA-1-81 and TRA-1-60) and expression of self-renewal genes (Oct-4, Nanog, SOX-2) to confirm the typical undifferentiated pluripotent stem cell phenotype	One iPSC sample (cryopreserved, fresh or fixed)	IF signal for all 6 markers
Induced pluripotent stem cells (iPSC) characterisation by	iPSC pluripotency assessment measuring mRNA expression of self-	One iPSC sample (cryopreserved)	Gene expression levels for all 3 markers

quantitative real-time PCR (qRT-PCR)	renewal genes SOX2, POU5F1 (OCT4) and NANOG by qRT-PCR	or fresh) or total RNA from one iPSC sample	
Induced pluripotent stem cells (iPSC) characterisation by 3-germ layer differentiation	iPSC pluripotency assessment by differentiation of the cells in all 3 germ layers (Endoderm, Mesoderm and Ectoderm).	One iPSC sample (cryopreserved or fresh)	Specific gene expression signatures for each germ layer

4. Tissue

Test	Scope	Min. input quantity	Readout
Histological QC (tumour/stroma/necrosis)	Measure the % tumour content	1 slide	% tumour assessment
snoRNA score assay	Assess the fixation time of tissue	50ng total RNA	Gene expression signature of specific snoRNA targets
Cold ischemia score assay	Assess the cold ischemia time of tissue	50ng total RNA	Gene expression signature of specific mRNA targets