

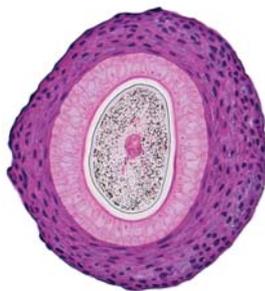
Plucked Hair Biomarker Platform

Gene and Protein Expression in Single Scalp Hairs

summary



Epistem provides a unique plucked hair biomarker platform to drug development companies targeting intracellular signalling pathways in oncology and other therapeutic areas. Plucked hair analysis offers a simple to apply and minimally invasive technique to access epithelial tissue drug induced changes. Effects on mRNA and protein expression levels can be analysed from plucked hairs and both can be applied to preclinical and clinical samples.



Transverse 3 μ m section of a single human hair, H&E stained

Plucked Hair Biomarkers

Plucked scalp hair is an ideal surrogate for measuring direct response to treatment. Highly vascularised, the hair follicle can respond to drug treatment within hours of exposure. Given this vascularisation, their epithelial nature and rapid rate of proliferation, the cells in the hair bulb at the base of the plucked hair and the outer root sheath are a highly relevant surrogate biomarker tissue for solid tumours and inflammatory diseases.

An important advantage of using hair in biomarker programmes is the minimally invasive nature of hair plucking, reducing patient discomfort and inconvenience and permitting multiple, independent samples to be collected over the treatment course. Within the clinic, it is also a relatively simple and inexpensive tissue sampling technique, with few ethical issues.

Sample Preparation

The scalp is the preferred source of plucked hair as most hairs in this region are in the anagen (active growth) phase of the hair cycle. Plucked hairs are received by Epistem either in RNeasy[®] for gene expression analysis or as fixed samples for immunohistochemical (IHC) analysis.

Ex vivo Hair Culture

Epistem has developed a proprietary *ex vivo* culture technique to monitor the effects that drug exposure has on gene and protein expression. This assay provides valuable proof of concept biomarker data, prior to a more costly clinical trial.

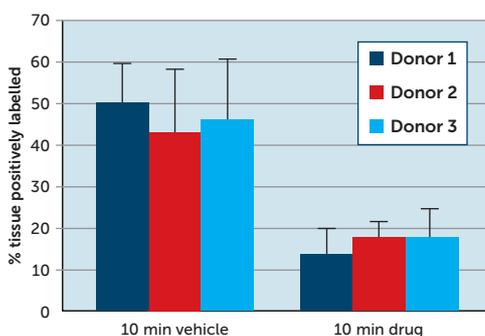
Epistem can source appropriate healthy donors for hair sampling. Donor hairs are plucked, immediately transferred to a maintenance medium and then exposed to drug at different concentrations. Once in culture, hairs can be collected at a range of time points for either mRNA or protein expression analysis. The technique shows good reproducibility and readouts can be quantified to show a dose response. This provides a cost effective assay to provide evidence of the utility of plucked hair as a surrogate biomarker tissue.

Gene Expression Analysis

Profiling of hairs is possible from both rodents and humans during preclinical and clinical studies. Small amounts of RNA (50-500 ng) are extracted from the bulbs at the end of anagen hairs. Representative cDNA is then prepared from the RNA, and gene expression levels determined by microarray analysis or by quantitative PCR (qPCR). Bioinformatic analysis is then conducted to identify drug-induced gene expression changes in hairs.

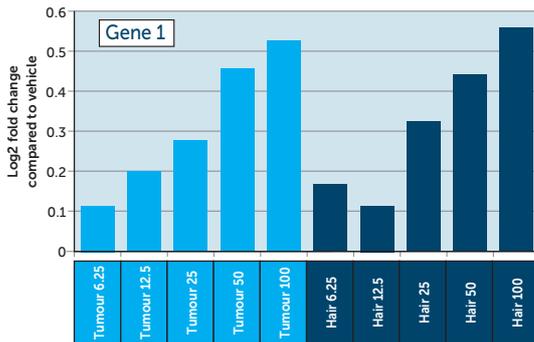
Epistem are also able to provide ready-to-use biomarker panels from our proprietary database containing information on key biochemical pathways that include:

- Notch
- PI 3-kinase
- Hedgehog
- c-Met
- EGFR
- WNT/ β -catenin
- Androgen receptor signalling
- Major inflammation pathways
- Major CNS disease pathways
- Major metabolic disease pathways

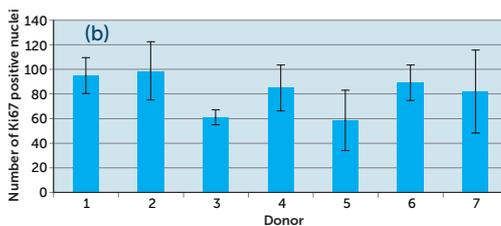
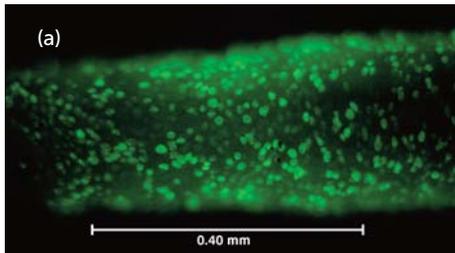


Ex vivo modulation of pERK1/2 (IHC assessment), in plucked hairs, with Tarceva[®]

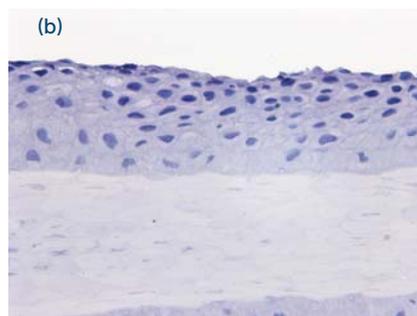
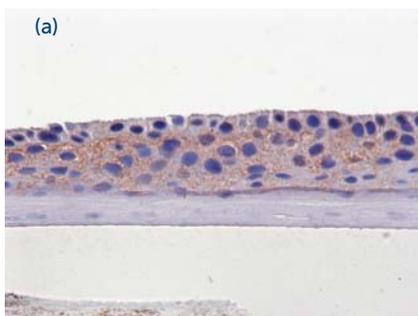
Plucked Hair Biomarker Platform



Comparison of gene expression profiles in hair and tumour samples following drug treatment



(a) Ki67 labelling in whole human hair which is then (b) quantified labelling in 5 hairs from each of 7 male donors. A 0.04 mm² area was analysed.



Phospho-AKT labelling in 3 µm longitudinal sections of human hair
(a) Phospho-AKT (b) Phospho-AKT labelling after pre-treatment with lambda phosphatase

Plucked Hair Immunohistochemistry

Epistem has developed techniques for IHC labelling of single plucked human hairs using fluorescence or DAB visualisation. We can detect and quantify protein expression and site-specific phosphorylation using either whole mounted or sectioned tissue. For each antigen, Epistem can establish a validation plan including optimisation of an antibody labelling protocol, levels of intra- and inter-donor variability and methods of quantification, as required. Our histology service currently utilises both Ventana 'Discovery Ultra' and Menarini 'Intellipath FLX' autostainers providing a reliable and reproducible service.

Clinically Relevant Surrogate Tissue

Epistem has analysed a range of markers relating to many clinically relevant cellular processes including:

- proliferation
- DNA damage and cell death
- hypoxia
- androgen response

Phosphorylated antigens can be analysed as direct targets to measure drug action (pharmacodynamics) both pre-clinically and as a surrogate tissue throughout a clinical trial. Hairs are collected globally at clinical sites and shipped to Epistem for analysis. Epistem can also provide complete sample collection kits, associated training and study long quality control support as part of a clinical trial, further information available upon request.

Whole Mount and Sectioned Hair

For the quantification of nuclear markers whole or sectioned hairs can be analysed. Whole mounted samples are labelled fluorescently, rapidly imaged and Z-sectioned using confocal scanning microscopy. Alternatively, formalin fixed paraffin embedded hairs can be sectioned longitudinally or transversely and used to quantify cytoplasmic, membrane or discrete nuclear expression changes.

Quantification

Labelling is quantified using manual or image analysis techniques as determined by a marker's expression pattern and a company's requirements. Our GCLP compliant Aperio Scanscope system is able to determine the labelling index (% labelled cells), labelling intensity and distribution of any marker examined.

Why Choose Epistem?

Our Expertise:

Epistem's Pharmacogenomics and Contract Research Divisions provide high quality biomarker and personalised medicine information to pharmaceutical and biotechnology companies from as little as a single hair. We specialise in advancing drug development programs for oncology, inflammatory and fibrotic disease indications through our innovative plucked hair analysis and laser capture microdissection techniques as well as offering GCLP accredited laboratory gene and protein expression as well as DNA genotyping services. In addition to our expertise in assessing limited quantities of RNA, we also have extensive experience in developing patient stratification companion diagnostic assays using our point of care Genedrive[®] platform.

Outstanding Service:

Epistem's high level of expertise and customer focus has led to an enviable track record and impressive repeat business ratio. Epistem has worked with over 200 pharmaceutical and biotechnology companies and has developed long term collaborative relationships with several market leaders.

Quality Management:

Epistem's pharmacogenomics and contract research laboratories are GCLP accredited. Our ISO 13485 certification is for the design, development, manufacture and distribution of molecular diagnostic instruments and molecular IVD assays.