Biomarkers in Cell Culture

If cell culture samples are used in assay systems which have been validated for the determination of biomarkers in serum and plasma, several aspects have to be taken into consideration.

An assay system validated for serum / plasma may be affected by sample material generated from cell culture.

To examine whether the assay system is influenced by cross-reactions or interferences induced by the components used, it is recommended to determine both the medium and all further substances in the assay. FCS in particular may crossreact with antibodies in the test or the media can affect the antibody response.

It is recommended to use a culture medium comprising the following components:

- DMEM or equivalent commercial medium
- Fetal bovine or calf serum or serum substitutes (FCS-free medium)
- L-Glutamine or ascorbate
- Antibiotic

To exclude the influence of FCS, it is recommended to use FCS-free medium, if possible.

Testing of medium, medium additives, and other buffers used

- **Buffer**: e.g. elution buffers which have been used to elute a substance from a gel or tissue
- **Matrix**: e.g. serum-free medium, serum substitutes
- Testing of **medium combination**:
  1. Medium
  2. Medium including all additives, but no FCS or alternative matrix
  3. Medium including all additives and FCS or alternative matrix (complete medium)
  4. Only FCS or corresponding alternative matrix
- **Recovery**:
  Add standard material/substance to the complete medium (spiking) to determine recovery;
  e.g. at a ratio 1:5, test 20% of the highest kit standard and 80% of complete medium in the assay
- **Linearity**:
  Measure dilutions of the spiked medium to control linearity:
  a) by using dilution buffer or zero standard from the kit at a ratio 1:2 and 1:4
  b) by using medium at a ratio 1:2 and 1:4
- **Standard curve (optional):**
  Dilute stock standard or the highest standard contained in the kit with complete medium and perform measurement

Specific recommendations

Assay	Analyt	Comments to the test procedure
BAP	Bone Alkaline Phosphatase	The Alkaline Phosphate is membrane bound and can be measured in cell lysate.
Osteocalcin	Intact Osteocalcin	The cells can be grown in serum containing media, but osteocalcin must be harvested from tissue culture supernatant that is serum-free.
CICP / PICP	C-terminal Propeptide of Type-I-Collagen (CICP)	The cells can be grown in serum containing media; however, to measure the parameters serum-free tissue culture supernatant has to be used.
DPD	Desoxypyridinolin-Crosslinks	Bone collagen specific – direct measurement of bone resorption. The cells can be grown in serum containing media; however, to measure the parameters serum-free tissue culture supernatant has to be used.
PYD	Pyridinolin-Crosslinks	
NTX	alpha-2 (I) N- telopeptide	
Helical Peptide	Helical Peptide	First results show that the ELISA is suited for the determination of the helical Peptide for in vitro analyses. This test method is especially advantageous because it requires small sample quantities and the curve covers a large range of the sample concentrations (30 fold of the CTX-beta in vitro Assays). The background concentrations through the medium are very low.
TRAP5b	Tartratresistent acid phosphatase 5b	Measurement of the osteoclast activity.

Measurement of BAP and Osteocalcin in cell culture

Additionally, 50 nM Vitamin D3 is necessary to generate measurable quantities of osteocalcin. The cells can be grown in serum containing media, but osteocalcin must be harvested from tissue culture supernatant that is serumfree, because the antibody will also detect this analyte in serum.

Bone alkaline phosphatase is membrane-bound and it has to be measured in culture from cell lysate. Cell culture media should not be treated with ion chelators like EDTA or citrate because of an inhibition of the BAP enzyme activity.

After the incubation the cell layers should be washed twice with saline and scraped into TMN buffer solution (20 mM Tris-HCl, pH 7.4; 2 mM MgCl2; 150 mM NaCl) uising a stir stick. The number of cells has to be determined (e.g. Coulter cell counter Model F) before the cells become solubilized by the addition of Triton X-100 to a final concentration of 1 %. Centrifugate the samples at 70,000g for 60 min and examine the aliquots of the supernatant for alkaline phosphatase activity.

Sheep and human cells will be fine for this purpose.

Measurement of CICP / PICP in cell culture

The assay is suitable for the measurement of cell culture supernatant from cells producing type-I collagen. The METRA® CICP Test can be used for the determination of the collagen production level of skin fibroblasts and bone cells.

It is recommended, that the supernatant of these cultures contains serum-free medium. Bovine serum in the medium may contain bovine CICP, which cross-reacts with the antibody in the kit. This would result in erroneously increased values.

In a confluating cell culture at an optimal collagen production level, the amount of CICP in the supernatant is approximately 20 – 50 ng/ml or slightly below a normal human serum sample. Dilute the cell culture supernatant 1:12 (like a normal serum sample) and calculate the final concentration according to the dilution. If the CICP concentration of the 1:12 dilution is below the detection limit it is possible to choose a 1:6-dilution.

References for BAP, PICP, OSTEOCALCIN in cell culture

Kaspar D, Seidl W, Neidlinger-Wilke C, Ignatius A, Claes L(2000) Dynamic cell stretching increases human osteoblast proliferation and CICP synthesis but decreases osteocalcin synthesis and alkaline phosphatase activity. J Biomech 33, 45-51.

Martínez ME, Medina S, Del Campo MT, et al. (1998) Effectof polyethylene on osteocalcin, alkaline phosphatase andprocollagen secretion by human osteoblastic cells.Calcif Tissue Int 62, 453-456.

Sell S, Gaissmaier C, Fritz J et al. (1998) Different behaviorof human osteoblast-like cells isolated from normal andheterotopic bone in vitro. Calcif Tissue Int 62, 51-59.

Siggelkow H, Niedhart C, Kurre W, et al. (1998) In vitro differentiation potential of a new human osteosarcoma cell line(HOS 58). Differentiation 63, 81-91.

Siggelkow H, Rebenstorff K, Kurre W, et al. (1999) Development of the osteoblast phenotype in primary human osteoblasts in culture: comparison with rat calvarial cells in osteoblast differentiation. J Cell Biochem 75, 22-35.