



## Multiplex analysis of released cytokines and their measurements in small volumes

Multiplex tests allow simultaneous detection of analytes. Their main advantage is very low need for the sample volume. Our aim was to generate a platform for analysis of activity of ADAM10 and ADAM17 metalloproteinases. Since ADAM10 and ADAM17 share some of their substrates, we have to measure several substrates in the same sample. Moreover, these enzymes shed ligands and as well as their receptors, and therefore concentrations of both ligand and receptor have to be measured. We prepared a platform where TNF receptor – soluble form, sMet-soluble form of HGF receptor, Amphiregulin – ligand of EGFR, Betacellulin – ligand of EGFR, sIL6R – soluble form of IL6 receptor and TNF alfa can be assayed simultaneously. Besides ADAM10 and ADAM17 substrates, we can also measure HGF that is a ligand of sMet. During this work we tested the interaction of antibodies and recombinant standard proteins. We found a combination of antibodies that are not binding each other and can be mixed together in a kit. This kit was used at the Department of Biochemistry, University of Kiel, and the results that were obtained by this kit are part of manuscript: Müller M et al., A disintegrin and metalloprotease 10 (ADAM10) is a central regulator of murine liver tissue homeostasis. ONCOTARGET Volume: 7 Issue: 14 Pages: 17431-17441. Another customer is the laboratory of Dr, Střížovský at IOCHB. Our knowledge obtained during validation, calibration and testing of this kit was fully used when we prepared a multiplex assay for chicken cytokines for the Biopharm company.

**Conclusions:** Our multiplex assay kit is ready, validated, and no other subject offers such kit for use in mouse research. This kit is not listed in any company offering the Luminex technology. Our kit is used in the Czech Centre for Phenogenomics, and we offer multiplex detection to our customers on commercial bases.

To get more information on this project and/or to purchase a nonexclusive license for the prototype of simplified, economical and accelerated analysis of RNA from low cell numbers, contact the Centre for Technology Transfer, IMG AS CR, Vídeňská 1083, 14220 Prague 4, Czech Republic; Tel. 420-241 063 227 or 420-602 892 876, e-mail: [ctt@img.cas.cz](mailto:ctt@img.cas.cz)