HIGH GRADIENT MAGNETIC SEPARATION (MACS) FOR ISOLATION AND MODIFICATION OF BIOMOLECULES

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INTRODUCTION: High gradient magnetic separation is not only applicable to cells, but also offers advantages in the isolation of biomolecules, such as nucleic acids or proteins (see Fig. 1). The small size of the particles involved gives rise to fast binding kinetics similar to a solution state reaction, which in turn reduces the time of separation. Low nonspecific binding as well as rigorous washing steps on the column lead to high purity products which can be used after elution or even processed further on the column. On-column reactions would add the benefits of solid phase reactions such as easy exchange of reaction media and efficient removal of excess reagent to the MACS system. The small void volume (25µl) of the µMACS column allows for economic use of reagents. To extend the possibilities of on-column reactions further, a heated magnet (ThermoMACS) was developed in order to enable reactions above room temperature. The following investigations were performed to evaluate in principle the possibilities of on-column modifications of magnetically immobilized biomolecules.

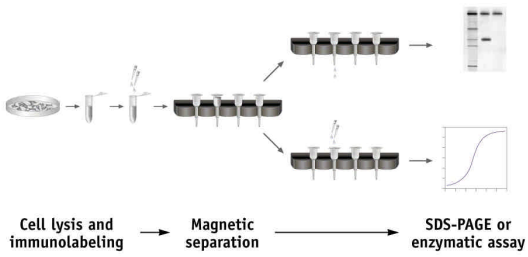

Fig. 1: Example for a reaction on the column: enzymatic activity assay after immunoprecipitation.

METHODS: Restriction digestion on the column: 5' biotinylated DNA (700 bp PCR product) is isolated with µMACS Streptavidin Kit. Restriction enzymes EcoRI, HindIII, SacI, SalI are used to cut the DNA on-column in a ThermoMACS (1 h / 37° C). After elution of the unbiotinylated fragment, release reagent is applied and the biotinylated fragment is isolated.

Activity of enzymes immobilized on the column after immunoprecipitation with µMACS Protein G or –A MicroBeads: An immunoprecipitation from Jurkat cell lysate is performed with anti v-src antibody (clone 327) and µMACS protein G MicroBeads. For assessing enzyme activity a nonradioactive kinase assay (Roche) is used. The substrate is applied to the column where the immunoprecipitated enzyme is magnetically immobilized. After elution the degree of phosphorylation is quantified by ELISA according to protocol.

cDNA synthesis on the column: mRNA is isolated from Jurkat cells with the µMACS mRNA Isolation Kit. Instead of eluting the mRNA according to protocol, a cDNA synthesis mix containing reverse transcriptase and dNTPs is incubated on the column in the ThermoMACS (50 min / 37° C). After RNAseH digest and release from the beads the cDNA is eluted.

RESULTS: Restriction digestion on the column: In order to show that efficient enzymatic reactions of magnetically immobilized molecules on the column are possible, digests with several restriction enzymes were performed on a biotinylated 700 bp PCR product. The amount of enzyme needed is the same as in a corresponding digest in a tube. Unbiotinylated and biotinylated fragments are eluted separately (see Fig. 2).

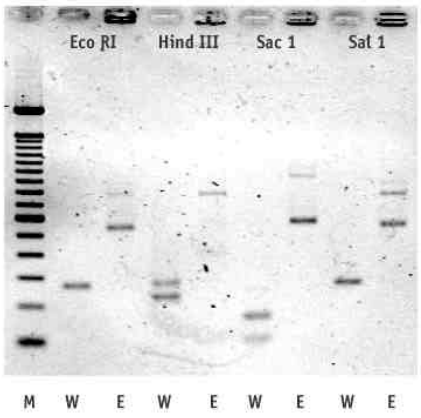

Fig. 2: ***Restriction digestion of 700bp DNA.*** *Agarose gel electrophoresis of the isolated fragments. Lanes W, unbiotinylated fragments; lanes E, biotinylated fragment eluted after release. Used enzymes: see above lanes.*

Activity of immunoprecipitated enzyme immobilized on the column: A kinase (81 kDa) could be isolated from Jurkat cells. A peptide substrate for v-

src was incubated on the column. ELISA of the eluted substrate showed that the peptide was phosphorylated.

cDNA synthesis on the column: mRNA from Jurkat cells was used for synthesizing cDNA. All steps were performed on the column in a heated magnet. The final eluate contained cDNA, which was confirmed by agarose gel electrophoresis.

Digestion experiments with DNAse and RNAse showed that the nucleic acid isolated is cDNA and not mRNA. A PCR amplification of housekeeping gene GAPDH showed that the resulting products were amplified from cDNA and not from genomic DNA, which would give larger PCR products with the used primer pair due to an intron present.

DISCUSSION & CONCLUSIONS: The examples above show that MACS enables not only fast and highly pure isolations of biomolecules, but also can be utilized to facilitate subsequent reaction steps by performing them on the column. The µMACS columns have a void volume of 25 µl which serves as the reaction volume. Through capillary forces the column matrices do not run dry, but stay filled even during prolonged incubation steps. Together with easy washing steps and exchange of reagents inside the matrix simply by applying the appropriate solutions, this contributes to the suitability of the columns as convenient reaction vessels. The ThermoMACS further broadens the range of possible applications by enabling reactions above room temperature.

In summary, the MACS system offers the possibility to switch between solid phase style and solution style steps simply by reversible magnetic immobilization on the column or use of the colloidal solution. So the benefits of both worlds can be used depending on the application. Isolations of biomolecules are done in the colloidal state which offers the advantage of fast binding kinetics. Modifications of isolated molecules can be done on-column, which offers the advantages of a small reaction volume for economic reagent use and efficient washing steps without the danger of losing precious material.