

## Protein extraction from mouse soft tissues

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#### CONTEXT

Immunotherapix is a start up company dedicated towards the development of innovative biologicals for the treatment of anti-inflammatory diseases.

To elucidate the anti-inflammatory mechanisms in the preclinical we use mouse models of inflammatory diseases. We analyze tissue and organs samples of injected mice with and without treatment via western blot for the presence or absence of biomarkers for different types of inflammations and immunoregulation. Currently we are extracting proteins either via the Precellys or Minilyls bead beater or directly in the tube with a motorised pestle [1].

#### MATERIAL

- Minilyls homogenizer.
- Precellys kit: 03961-1-004 (VK05 glass beads).
- Samples: ~50 mg of organ samples (spleen, colon, lung) were flash-frozen in liquid nitrogen and stored at -20°C. Samples were unfreezed on ice before the lysis.
- Buffer: 250µl of RIPA buffer supplemented with 10% Protease inhibitor (Sigma P8340).

#### PROTOCOL

- Minilyls: 5000 rpm, 3x60sec (in ice between each cycle).
- After lysis, the tubes are centrifuged at 10000 rpm for 10 min. 100µl of the thus obtained supernatant were used for the further analysis.

- The protein content of the samples was estimated with the QuantiT Protein assay kit Invitrogen Q33211, using a 100-fold dilution of the original sample.



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#### RESULTS

The protein recovery was estimated as mg protein in solution standardized to the total weight of the lysed tissue.

As reported in the table 1, we obtained excellent results from different mice tissues using the Minilyls apparatus for tissue lysis.

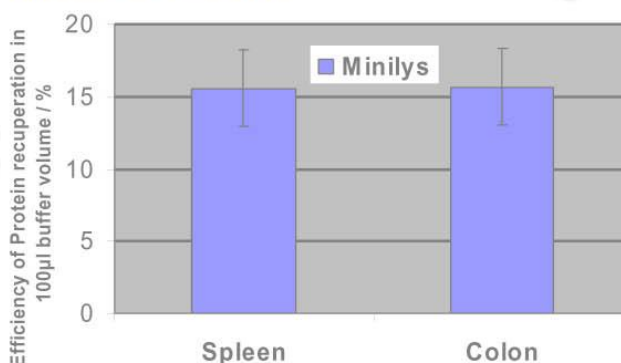


Table 1: Protein recovery in 100µl buffer volume compared to the initial weight of the lysed spleen and colon tissue obtained with Minilyls.

We also obtained goods results using the Precellys apparatus for tissue lysis.

In any case, Precellys based extraction was much more efficient than any other previously used method in our laboratory.

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#### CONCLUSION

In our laboratory the Precellys based lysis of tissue is now our standard procedure for the preparation of protein extracts from tissues. We also use **Precellys** for the preparation of RNA and genomic DNA and we were always completely satisfied with its performance. **Minilyls** is a real affordable alternative for laboratories with a low or medium throughput of samples. If the heating of the samples due to the longer lysis time is not from importance, this apparatus can prepare protein extracts from tissues with a very high efficiency.

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