Application Note

Sample Preparation for Radio Immunoassay with Vivaspin 2 Ultrafiltration Devices

Christian Albers, Justus Beike, Matthias Lehr.

Institute for Pharmaceutical and Medicinal Chemistry and Institute for Forensic Medicine, University of Muenster, Germany.

Introduction

Raising antibodies against small molecules like drugs (MW < 1000 kDa) requires the immunisation of animals e.g. rabbits with a specific immunogen. To obtain antiserum of high quality, it is necessary to closely monitor the immunisation. For this, 36 days after immunisation and subsequently every 14 days, a blood sample is extracted from the host to analyse the produced antibodies. A common method for controlling the immunisation is performing a radio immunoassay in which the host's antiserum is diluted (1:100, 1:250 and 1:500) and incubated with a radioactive tracer. The tracer molecules reacts with the antibodies to form an antigen-antibody-complex. To measure the radioactivity of this complex, unbound tracer must be removed.

Usually the complex will be precipitated with $(NH_4)_2SO_4$ or trichloroacetic acid, centrifuged and the supernatant containing unbound tracer discarded. The pellet containing the bound tracer can be further utilized for measurements revealing the antibody specificity.

During precipitation however, varying amounts of unbound tracer molecules can be co-precipitated along with the complex, leading to irreproducible measurements.

For this reason we used Vivaspin 2 ultrafiltration devices with a 50 kDa MWCO PES membrane to separate the complex from unbound tracer. Using this method, the complex could be easily freed from the disturbing unbound tracer and measured without further precipitation.

Methods

100 µl antiserum was incubated with 100 µl tracer (tritiated psilocin, 2000 counts per minute) in a vial (1.5 ml). The solution was refilled into a Vivaspin 2 device (50 kDa, PES), the vial rinsed with 500 µl PBS buffer (which was added to Vivaspin 2) and centrifuged (10 min, 2889 x g). After washing with 500 µl PBS buffer (centrifugation 10 min, 2889 × g), the radioactive complex was regained by reverse spinning.

Radioactive measurement

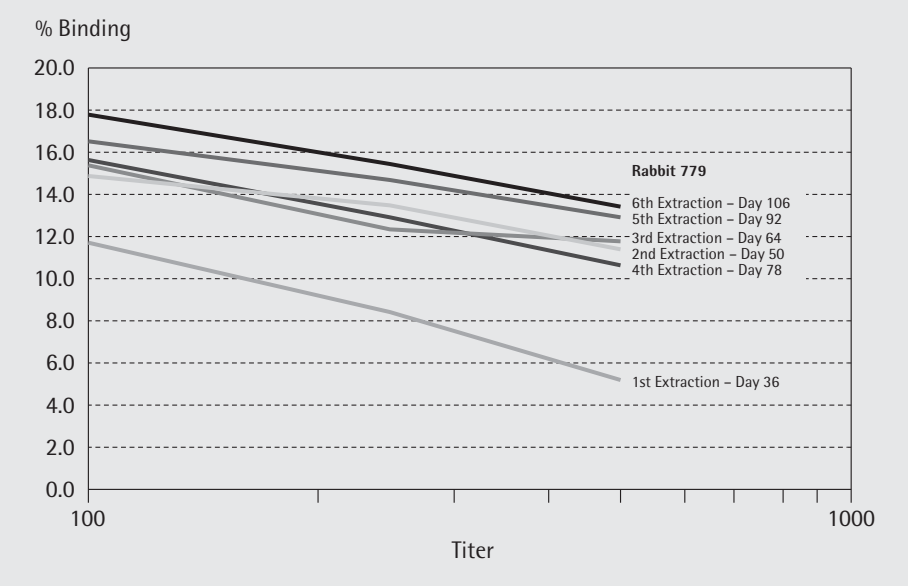
The radioactive complex concentrate was filled into a scintillisation vessel, the recovery cap was rinsed twice with 150 µl PBS buffer and added to the concentrate in the vessel. The vessel was then measured in a ß-counter.

Results
The immunisation trend is shown in different dilutions (1:100, 1:250, 1:500) of the antiserum for one rabbit in figure 1.

Conclusion
The results presented here show that Vivaspin 2 is a good alternative for sample purification in a radio immunoassay. The filter with the 50 kDa membrane is suitable to restrain an antigen-antibody-complex while allowing the radioactive tracer with a molecular weight below 1 kDa to pass the membrane.

After reverse spinning the retentate freed of the unbound radioactive tracer can be measured, leading to consistent radio immunoassay results. In our case an increase in antibody specificity during the immunisation period could be observed.

Figure 1: Immunisation trend showing different dilutions (1:100, 1:250, 1:500) of blood samples analysed by radio immunoassay between the first extraction (36 days after immunisation) and the 6th extraction (106 days after immunisation).



Sartorius AG
Weender Landstrasse 94–108
37075 Goettingen, Germany

Phone +49.551.308.0
Fax +49.551.308.3289
www.sartorius.com

USA +1.800.3687178
UK +44.1372.737100
France +33.1.69192100
Italy +39.055.634041
Spain +34.91.3586100
Japan +81.120.82.5533

Publication No.: SLL4005-e06111
Order No.: 85030-532-28