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Application Note

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Free Label Removal with Vivaspin® 2 Prior to Radioimmunoassay

Christian Albers, Justus Beike, Matthias Lehr.
Institute for Pharmaceutical and Medicinal Chemistry and Institute for Forensic Medicine,
University of Muenster, Germany.

Abstract

In life science research, molecular labels are often used to aid purification, detection or quantification of the protein of interest. For reliable and accurate results, it is important to ensure that protein samples do not contain any unbound label following labeling reactions. However, during conventional techniques such as precipitation, free label removal can be inefficient, due to co-precipitation. In this study, we demonstrate reliable removal of unbound radiolabel from an antigen-antibody complex by fractionation using centrifgual ultrafilters. Compared to precipitation techniques, Vivaspin® 2 enabled efficient and simple separation of unbound label from the target molecule, prior to radioimmunoassay.

Introduction

Raising antibodies against small molecules like drugs (MW < 1 kDa) requires the immunization of animals such as rabbits with a specific immunogen. To obtain antiserum of high quality, it is necessary to closely monitor the immunization. For this, 36 days after immunization and subsequently every 14 days, a blood sample is extracted from the host to analyze the produced antibodies. A common method for monitoring the immunization is to perform a radioimmunoassay in which the host's antiserum is diluted (1:100, 1:250 and 1:500) and incubated with a radioactive tracer. The tracer molecules react with the antibodies to form an antigen-antibody complex. For accurate radioactivity measurements 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 is then 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 measurements which are not reproducible.

For this reason we used Vivaspin® 2 ultrafiltration devices with a 50 kDa MWCO PES membrane to separate an antigen-antibody complex from unbound tracer. Using this method, the complex could be easily fractionated from the interfering unbound tracer and measured without the need for precipitation.

Materials and Methods

Radiolabeling and Free Label Removal
100 µL antiserum was incubated with 100 µL tracer (tritiated psilocin, 2,000 counts per minute) in a vial (1.5 mL). The solution was applied into a Vivaspin® 2 device (50 kDa MWCO PES), the vial rinsed with 500 µL PBS (which was also then added to Vivaspin® 2) and centrifuged (10 min, 2,889 *g*). After performing a wash step with 500 µL PBS (centrifugation for 10 min, 2,889 *g*), the radioactive complex was recovered from Vivaspin® 2 by reverse spinning.

Radioactive measurement
The radioactive complex concentrate was filled into a scintillation vessel, and the Vivaspin® recovery cap was rinsed twice with 150 µL PBS and pooled with the concentrate in the vessel. Radioactive measurement of the vessel contents was performed in a ß-counter.

Results

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

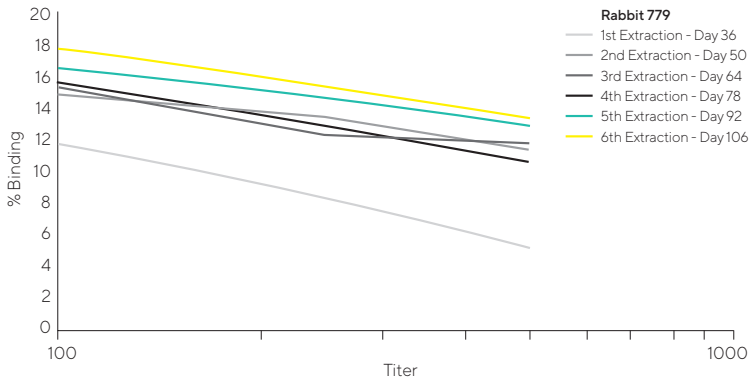
Conclusion

The results pres ented here show that Vivaspin® 2 is a good alternative for sample preparation prior to radioimmunoassay. The ultrafiltration device with 50 kDa MWCO PES membrane is suitable to retain an antigen-antibody complex while allowing the radioactive tracer with a molecular weight below 1 kDa to permeate the membrane.

Furthermore, the reverse spin feature simplified recovery of the retentate, which - free of unbound radioactive tracer - could be used for radioimmunoassay measurements with increased accuracy and consistency. In our case, an increase in antibody specificity during the immunization period could be observed.

Figure 1.

Immunization trend showing different dilutions (1:100, 1:250, 1:500) of blood samples analyzed by radioimmunoassay between the first extraction (36 days after immunization) and the 6th extraction (106 days after immunization).



Germany
Sartorius Lab Instruments GmbH & Co. KG
Otto-Brenner-Strasse 20
37079 Goettingen
Phone +49 551 308 0

USA
Sartorius Corporation
565 Johnson Avenue
Bohemia, NY 11716
Phone +1 631 254 4249
Toll-free +1 800 635 2906

For further information, visit
www.sartorius.com

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