Benchmarks

D. Stuber and K. Henco. 1994. 6xHis-Ni-NTA chromatography as a superior technique in recombinant protein expression/purifica-

3. Galibert, F., E. Mandart, F. Fitoussi, P. Tiol-
lais and P. Charnay. 1979. Nucleotide se-
quence of the hepatitis B virus genome (sub-

fied human immunodeficiency virus tat-en-
coded protein: trans-activation requires
mRNA synthesis. Proc. Natl. Acad. Sci. USA
86:821-824.

5. Smith, D.B. 1993. Purification of glutathione-
S-transferase fusion proteins. Methods Mol.
Cell Biol. 4:220-229.

step purification of polypeptides expressed in
Escherichia coli as fusions with glutathione S-

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Table 1. Procedure for Producing Moderate Amounts of Polyclonal Antibodies in Mice

1. Inject pristane (0.5 mL) (ICN, Costa Mesa, CA, USA) intraperitoneally into a
mouse.

2. Wait 10–15 days before immunizing the mice in either of two ways: (i) mix 100
µL antigen (containing 100 µg of protein or peptide conjugated with carrier pro-
tein) with 100 µL of MPL+TDM Emulsion (Ribi ImmunoChem Research, Hamilton,
MT, USA) and inject intraperitoneally; or (ii) emulsify antigen with complete
Freund’s adjuvant and inject subcutaneously at multiple sites.

3. Wait 15–20 days, then boost the immunized mouse with antigen, which can be
mixed with MPL+TDM Emulsion as described in step 2 or emulsified with in-
complete Freund’s adjuvant and injected intraperitoneally.

4. Boosting is repeated one more time after another 15–20 days (as described
above).

5. Test for antibody titer by either enzyme-linked immunosorbent assay (ELISA) or
Western blot with a small amount of blood from the tail vein of the immunized
mouse. If the blood sample gives a good titer, proceed to step 6. Otherwise, ei-
ther repeat the immunization protocol in step 3 or terminate the procedure if the
antigen is suspected to be nonimmunogenic.

6. Prepare about 0.5 million to 1 million myeloma cells for each mouse by washing
them with phosphate-buffered saline (PBS) to remove serum. Appropriate type
of myeloma cells should be used for the particular strain of immunized mice.
For example, SP2/0 myeloma cells (Catalog No. CRL 1581; ATCC, Rockville,
MD, USA) should be used for Balb/c mice. Inject the washed myeloma cells in-
traperitoneally to induce ascites fluid production.

7. The mouse will start accumulating ascites fluid in about a week. The ascites
fluid may be collected daily for about 5–8 days.

Table 1. Procedure for Producing Moderate Amounts of Polyclonal Antibodies in Mice

Antibodies are important tools in
biomedical research and diagnostics.
Most antibodies are generated either as
polyclonal antibodies in rabbits or as
monoclonal antibodies in mice. Mice
have not been used for polyclonal anti-
body production because they produce
only limited amounts of serum. How-
ever, antibody production in rabbits re-
quires more antigen. Since different
rabbits produce different titers or prop-
ties of antibodies, it is a common
practice to immunize several rabbits at
the same time to minimize this varia-
tion. This requires even more antigen
and increases the cost of antibody pro-
duction. If one can increase the amount
of polyclonal antibodies produced by
immunized mice, their use is more
economical. Furthermore, the same

Figure 1. Reactivity of serum or ascites from same mouse with peptide antigen. ELISA was per-
formed as described by Liang et al. (2).
immunized mice can be used for the production of monoclonal antibodies if desired.

Harlow and Lane (1) have discussed the possibility of obtaining polyclonal antibodies from mice, but they did not provide a detailed procedure. Using their approach, we have developed a simple procedure that can produce moderate amounts of polyclonal antibodies in mice. This procedure is based on the fact that immunized mice can be induced to produce a significant amount of ascites fluid that contains the desired antibodies. Thus, one is not limited by the low supply of serum in each mouse. Typically, we can obtain 5–15 mL of ascites fluid from a single immunized mouse. If the antibodies have good titers, this amount should be sufficient for most purposes. And if the amount of antigen is not a limiting factor, several mice can be immunized at the same time to increase the amount of available antibodies and to minimize variation among individual mice. The procedure that we developed is outlined in Table 1.

The following example illustrates how this procedure is used to generate an anti-peptide antibody. Five Balb/c mice were injected with the keyhole limpet hemocyanin (KLH)-coupled peptide antigen. When tested by ELISA, two of these mice showed good titers. Ascites were subsequently induced in both mice by injecting SP2/0 cells. These ascites have antibody titers similar to those found in sera, as shown in Figure 1. The titers and amounts of sera and ascites obtained are summarized in Table 2.

One of these two mice was injected with an additional dose of antigen subcutaneously at the first sign of weakness (about 3 days after ascites collection started). Three days later, the animal was sacrificed, and its spleen cells were used to generate monoclonal antibodies. Two monoclonal antibodies were thus generated. This experiment demonstrates the feasibility of obtaining both polyclonal and monoclonal antibodies from the same animal. However, when generation of monoclonal antibodies is also desired, one must compromise on the amount of ascites collected because the last dose of antigen must be administered before the mouse shows signs of weakness.

In summary, we have developed a convenient procedure for obtaining sufficient amounts of polyclonal antibodies from mice. In addition, we showed that it is feasible to obtain both polyclonal and monoclonal antibodies from the same immunized mouse. This simple procedure makes antibody production more affordable. This in turn should promote wider use of antibodies in many studies, which will otherwise be impossible because of either antigen or budget constraints.

**REFERENCES**


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Detection of specific nucleotide sequences in DNA is frequently achieved by hybridizing radioactive probes to DNA that has been transferred to nitrocellulose or nylon and denatured by the technique described by Southern (6). Alternative probe labels have been developed that replace the use of radioactive isotopes (3). Procedures for probing DNA sequences in agarose gels have also been described; however, these methods require the use of radioactive isotopes (4).

We have developed a method for immobilizing and detecting specific DNA sequences that requires neither the use of radioactive isotopes nor the transfer of DNA to a support like nitrocellulose or nylon. Briefly, the method involves the use of a 5'-biotinylated oligonucleotide to probe denatured DNA in agarose gels and detection of the probe with a streptavidin-alkaline phosphatase conjugate that is subsequently revealed by exposure to a chromogenic substrate.

This method should be of interest to those laboratories that do not have the license or facilities to work with radioactive isotopes, to personnel that have no training in handling isotopes and to those wanting to avoid the storage and disposal problems that accompany the use of radioisotopes. It should also be of interest to those that want to avoid the labor and expense associated with Southern transfer procedures and autoradiography. Furthermore, this method has been used with great success in our undergraduate instructional laboratories, and therefore it may be attractive to those involved in teaching applied molecular biology.

The specific method developed for our research involved electrophoresing restriction-digested and undigested phage λndr (2) DNA in a 0.8% agarose gel (6.5 × 8.0 cm) prepared with TAE buffer (5) and containing ethidium bromide at 0.5 μg/mL. After

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**Table 2. Reactivities of Serum and Ascites with Peptide Antigen**

<table>
<thead>
<tr>
<th>Mouse No. 1</th>
<th>Mouse No. 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Volume (mL)</strong></td>
<td><strong>Titer</strong></td>
</tr>
<tr>
<td>Serum</td>
<td>0.2</td>
</tr>
<tr>
<td>Ascites</td>
<td>7.0</td>
</tr>
</tbody>
</table>

*Serum or ascites dilution factors at which signals produced with peptide antigen are greater than background levels in ELISA.