patients (Figure 2). Then, three specimens with a 200-bp product were analyzed employing a LightCycler®-t[9;22] Quantification Kit™ (Roche Molecular Biochemicals, Mannheim, Germany). In these specimens, using two probes specific for human glucose-6-phosphate dehydrogenase (G6PDH) transcripts and for bcr-abl fusion transcripts, the bcr-abl mRNA:G6PDH mRNA ratio was estimated to be 0.08, 0.12, and 0.29, respectively. The results of amplification of this fusion mRNA were the same for RNAs extracted from the leukocytes on PET fiber after one day of storage as for those extracted after two weeks of storage.

No notable difference was found in the expression of either GAPDH mRNA or bcr-abl mRNA between filters stored in ethanol for two weeks at 4°C and processed filters fixed with ethanol after one day of storage.

RT-PCR analysis for the bcr-abl gene is conducted routinely because of its high degree of sensitivity and reliability (3,4,6,8), instead of other molecular methods such as quantitative Southern blotting, FISH, quantitative western blotting, and cytogenetic analysis of bone marrow cells.

Our results showed that EDTA-anticoagulated blood (about 1 mL) obtained by our filtration method could be used for RNA extraction and gene analysis. The results also showed that the amount of RNA obtained from leukocytes was sufficient and the quality of RNA suitable for RT-PCR analysis. Our specimens remained free of denaturation and degradation for two weeks of storage at 4°C. Our method thus promises to enable simple, rapid, and safe testing of biohazardous blood specimens in the medical laboratory.

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Received 2 March 2000; accepted 3 January 2001.

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Elimination of Reducing Agent Facilitates Quantitative Detection of p53 Antigen

BioTechniques 30:960-963 (May 2001)

Immunoprecipitation of an antigen serves as a very useful tool for the analysis of proteins and protein-protein interactions. This is most commonly achieved by incubation of the antibody with cell or tissue extract followed by selective entrapment of antigen-antibody complexes using immobilized protein A and/or protein G (4). Immunoprecipitated protein(s) are then solubilized by addition of a buffer containing SDS and a strong reducing agent such as β -mercaptoethanol or dithiothreitol, followed by boiling. This procedure causes rapid and irreversible denaturation of proteins, including the antigen and antibody molecules. As a result of such a treatment, the immunoglobulin (Ig) molecules involved in immunoprecipitation separate into the approximately 50 kDa heavy and 25 kDa light chains upon denaturing SDS-PAGE. Proteins in the gel are then typically electroblotted onto a nitrocellulose or a polyvinylidene difluoride (PVDF) membrane (7). After blocking free sites on the membrane, the antigen is detected by standard methods of a sensitive enhanced chemiluminescence technique using secondary antibodies conjugated with horseradish peroxidase. However, the immunoglobulin chains, especially the heavy chain, generate a strong signal by virtue of their cross-reactivity with the secondary antibody and are considered to be inevitable artifacts of the procedure. Unfortunately, this cross-reactivity poses a serious difficulty if the antigen under study, such as the extensively studied p53 tumor suppressor protein, migrates at a position very close to either of these chains. Two alternatives are currently under use to circumvent this problem. The first is to use secondary antibodies from a different, non-cross-reactive species. However, such antibodies of desired specificity are not always available, are usually unsuitable for quantitative immunoprecipitations, and may lead to er-

roneous interpretations in the event of weak cross-reactivity. Furthermore, the heavy chain molecules may locally saturate the binding sites on the membrane, leading to incomplete transfer of other protein molecules in the 50–55 kDa molecular weight range. Second, the antigen or the antibody could be tagged, either by radioisotopic or non-isotopic methods, or immobilized by coupling to beads. These procedures may not provide the most suitable alternatives because they are either more cumbersome or may affect the sensitivity of detection and quantitation. We report a quick and easy method of general significance for eliminating the interference of immunoglobulin heavy chains during the detection of antigens in the 50–55 kDa molecular weight range using p53 as an example.

The disulfide-linked subunits of the Ig molecule separate upon treatment with β -mercaptoethanol and are detected as two polypeptides of 50 and 25 kDa in a typical western blot analysis (Figure 1A, lane 1). This is the background signal generated because of the cross-reactivity of the Ig chains with the secondary antibody. However, nuclear extract probed under identical conditions indicates that the position of the signal corresponding to the p53 antigen is almost identical with that of the Ig heavy chain (Figure 1A, lane 3). When a p53 immunoprecipitate is separated and probed under identical conditions, it is apparent that the background signal corresponding to the immunoglobulin heavy chain overlaps completely with the signal corresponding to the p53 antigen (Figure 1A, lane 2). We circumvented this problem by omitting the reducing agent from the immunoprecipitate solubilization buffer. Under this condition, the Ig molecule migrates as though it would run in a native gel, as a tetramer of two heavy and two light chains that are held together by disulfide links, with an apparent molecular weight of approximately 160 kDa as judged by SDS-PAGE (Figure 1B, the top band in lanes 1–3). Densitometric analysis of the signals corresponding to p53 released from the anti-p53 immunoprecipitates in Figure 1B showed a linear dose-response curve, indicating that the release of antigen is quantitative (Figure 1C).

Immunoprecipitations are often carried out to study protein-protein interactions. The human RAD51 protein physically interacts with p53 (6). WTK1 cells express only mutant p53 (Met 273 Ile) (5,8,9). We wished to ascertain whether mutant p53 in WTK1 cells that was shown to exhibit only a wild-type conformation (1) can be quantitatively released from anti-RAD51 immunoprecipitates under identical conditions as described above. We detected p53 in anti-RAD51 immune complexes of WTK1 cells when we performed the immunoprecipitation reactions under non-reducing conditions. When we employed increasing amounts of nuclear protein extract in the anti-RAD51 immunoprecipitation reactions, increasing amounts of p53 were detected by immunoblot analysis using mouse monoclonal anti-human p53 antibody (Figure 2A, lanes 1–3). We found that heating at 95°C to release the antigen did not significantly increase the yield of immunoprecipitated p53 (Figure 2A, lanes 4 and 5). Moreover, heat treatment caused more smearing of the IgG signal, and, therefore, we chose not to use it. To eliminate the possibility of even the smallest amount of denaturation of the antibody and subsequent detection of heavy chain on immunoblots, we probed a duplicate immunoblot with anti-rabbit secondary antibody to visualize the antibody molecules used in the immunoprecipitation reactions. As seen in Figure 2B, we detected a signal corresponding only to the tetrameric Ig molecules (lanes 1–3). Upon boiling in the presence of β -mercaptoethanol, the heavy chains from the immune complexes migrate as monomers (Figure 2B, lane 4). The densitometric analysis of the p53 signals from lanes 1–3 in Figure 2A demonstrated the linearity of the procedure (Figure 2C). Thus, our method can be used to quantitatively elute and detect p53 in immunoprecipitates, and we also propose that this method may be used to analyze many other antigens in the 50–55 kDa range, provided they are not disulfide linked.

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Benchmarks

Figure 1. Detection of p53 in anti-p53 immunoprecipitates solubilized in a buffer lacking reducing agent. Nuclear extracts were prepared from 1×10^8 WTK1 human lymphoblasts as previously described (3). Protein extracts were dialyzed for 14 h at 4°C in 20 mM potassium phosphate, pH 7.4, 50 mM NaCl, and 10% glycerol. Protein quantitation was performed using the modified Lowry Assay (Bio-Rad Laboratories, Hercules, CA, USA) according to the manufacturer's description. (A and B) Nuclear protein (16–50 μg) from WTK1 cells were diluted in 600 μL Dignam buffer A (10 mM HEPES, pH 7.9, 1.5 mM $MgCl_2$, 10 mM KCl, plus 0.5 mM Pefabloc® SC and protease inhibitor cocktail (both from Roche Molecular Biochemicals, Indianapolis, IN, USA). For preclearing, 1 μL of 0.25 mg/mL mouse IgG2a (Sigma, St. Louis, MO, USA) was added to the extract and incubated at 4°C for 1 h, followed by the addition of 10 μL Protein A/G Plus beads (Pierce Chemical, Rockford, IL, USA) and incubation at 4°C for 1 h on a rotating shaker. The precleared extracts were incubated with 5 μL of 0.1 mg/mL Ab-5 conformation-specific anti-p53 antibody (Calbiochem-Novabiochem, San Diego, CA, USA) overnight at 4°C, followed by addition of 10 μL Protein A/G Plus beads and incubation for two additional hours at 4°C. The beads were then washed four times at 4°C in PBS containing 0.5% Nonidet® P-40 and collected by centrifugation at 4000× *g* for 5 min. Immune complexes were eluted from the Protein A/G plus beads by incubation at either 37°C for 10 min or by incubation at 95°C for 5 min in 40 μL of 2× SDS-PAGE sample buffer. After centrifugation at 12 000× *g* for 10 min, samples were electrophoresed on a 12% SDS-polyacrylamide gel and transferred to PVDF membranes (Millipore, Bedford, MA, USA) (2). The western blots were stained successively with mouse monoclonal Ab-6 anti-human p53 antibody (Calbiochem-Novabiochem) at 1:3000 dilution and HRP-conjugated secondary goat anti-mouse antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 1:10 000 dilution followed by detection using the enhanced chemiluminescence Supersignal® West Pico substrate kit (Pierce Chemical). (C) Densitometric analysis of p53 signal from Figure 1B. The signal intensities for p53 were quantitated using the ChemiImager™ 4400 Low Light Imaging System (Alpha Innotech, San Leandro, CA, USA) and AlphaEase™ software (version 5.0). Relative amounts of immunoprecipitation p53 protein detected by western analysis are plotted as a function of the amount of nuclear protein extract employed per reaction. Data points represent the mean of three experiments ± 1 SD.

Figure 2. Detection of p53 in anti-RAD51 immunoprecipitates solubilized in a buffer lacking reducing agent. Immunoprecipitation reactions and western blot analyses were performed as described in the legend to Figure 1 except that 50–200 μg protein were used. (A) Quantitative western blot to demonstrate the presence of p53 in anti-human Ab-1 RAD51 immune complexes. One microliter of 0.25 mg/mL rabbit IgG (Sigma) and 1 μL polyclonal rabbit anti-human RAD51 antibody (Calbiochem-Novabiochem) were used for preclearing and co-immunoprecipitation, respectively. (B) Western blot was performed as in Figure 2A, lanes 1–3, and stained with anti-rabbit secondary antibody only to visualize the rabbit anti-human RAD51 antibody used in the immunoprecipitation; lane 4: sample boiled in the presence of β-mercaptoethanol (β-ME). (C) Densitometric analysis of data shown in Figure 2A as described in the legend to Figure 1.

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The authors wish to thank Drs. Amy Kronenberg and Terumi Kohwi-Shigematsu for their support and encouragement. This work was supported by NASA grant no. T-964W to A.K., NIH grant no. R01 CA39681 to T.K.-S., and the United States Army Breast Cancer Research Project DAMD 17-98-1-8303 to S.G. Address correspondence to Dr. Sanjeev Galande, National Centre for Cell Science, NCCS Complex, Ganeshkhind Road, Pune 411007, India. e-mail: sanjeev@nccs.res.in

Received 12 September 2000; accepted 7 February 2001.

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Sterile Preparation of Antibiotic-Selective LB Agar Plates Using a Microwave Oven

BioTechniques 30:963-965 (May 2001)

LB agar plates are simple, but powerful tools in molecular biology that allow for the growth of bacteria that can then be used for a wide variety of applications. One of the most common applications for these plates is the selective growth of bacterial cells that have been transformed by a plasmid with an antibiotic-resistant gene linked to it (2). Selection is achieved by matching the antibiotic resistance of the transformed cell to the antibiotic added to the growth media.

Traditional methods for preparing LB agar plates require the media to be autoclaved as a means of sterilization and then enhanced with an antibiotic before pouring the media into plates (1). This method of media preparation can be inconvenient when the plates are needed for immediate use. Recently, a short protocol posted on the Internet (<http://www.moorhead.msus.edu/chastain/html/-lbplates.html>) suggested that the sterilization of media by autoclave could be replaced with a short round of heating the media in a microwave oven. Furthermore, a commercial vendor offers a product intended for preparation by this method (Immedia; Invitrogen, Carlsbad, CA, USA). To the best of our knowledge, there are no published data that substantiate this method. The following protocol explores the novel method of LB agar and antibiotic plate preparation that uses a microwave oven instead of an autoclave and rapidly produces quality plates without compromising their sterility.

We prepared aliquots of 35 g/L powdered LB agar with powdered antibiotic, either 100 µg/mL carbenicillin or ampicillin, or 50 µg/mL kanamycin (all products purchased from Sigma, St. Louis, MO, USA) in 50-mL sterile conical tubes, sufficient to make 200 mL molten agar solution for eight 100 × 15 mm plates. For each antibiotic condition, seven aliquots were prepared. One set of aliquots was used to prepare