Monoclonal Antibodies Specific for Globin Chains

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Six monoclonal antibodies specific for human globin chains are described. They are produced by stable clones obtained by raising hybridomas using cells of mice immunized with either adult or fetal hemoglobin. Characterization of the antibodies included testing against tetrameric human and other animal hemoglobins, isolated hemoglobin chains, and when indicated, cyanogen bromide fragments. Monoclonals 16-2 and 37-8 are $\alpha$-chain specific. Antibody 31-2 recognizes an antigenic determinant common to the $\alpha$ and $\beta$ subunits. Monoclonal 30-3 recognizes determinants best expressed in the $\alpha_2\beta_2$ tetramer. Antibody 45-1 recognizes a determinant common to $\beta$ and $\gamma$ subunits, while antibody 51-7 is $\gamma$-chain specific. None of the monoclonal antibodies recognizes mouse hemoglobin, and they display significant differences in binding to hemoglobins of various species. The species-specific reactions and the knowledge of the primary structures of globins allowed deductions about the antigenic sites recognized by two of the monoclonals (16-2 and 45-1). These antimoglobin monoclonal antibodies will provide useful probes for studying hemoglobin expression in vivo and in vitro.

**IMMUNOCHEMICAL** methods have provided useful tools in the investigation of the cell biology of human and animal hemoglobins.\(^1\)\(^8\) Past work has utilized monospecific conventional antibodies against normal or variant hemoglobins.\(^9\)\(^18\) The raising of hybridomas allows the acquisition of stable clones secreting monoclonal antibodies that can be obtained in pure form.\(^19\)\(^20\) We have previously described\(^21\) the production of one such monoclonal antibody, designated Hb $\beta$ 3-2, and have described a strategy whereby (a) the probable antigenic determinants recognized by the monoclonal antibody are deduced by comparing the primary structures of animal hemoglobins that are or are not recognized by the antibody, and (b) the deductions are tested by reacting the monoclonal antibody with human mutant hemoglobins bearing structural aberrations at the putative antigenic sites. The purpose of this article is to describe the production of six new monoclonal antibodies that are specific for hemoglobin chains and to describe their binding characteristics with hemoglobins and globin chains from various animal species.

**MATERIALS AND METHODS**

**Preparation of Hemoglobins**

Adult human hemoglobins were isolated by ion-exchange chromatography as previously described.\(^22\)\(^23\) Hb Niteroi\(^24\) and Hb Ocho Rios\(^25\) were isolated by chromatography on 2.5 x 45 cm columns of DEAE-Sephadex (A-50), using a linear gradient formed by gradual addition of 0.05 M Tris-HCl, pH 7.28 (limiting buffer) to a starting buffer of 0.05 M Tris-HCl, pH 7.95. Both buffers were 10 \(^{-1}\) M in KCN. Hemoglobins isolated by column chromatography were concentrated by ultrafiltration, dialyzed extensively against phosphate-buffered saline (PBS), and stored at –70°C. Hemoglobins\(^26\) from an adult baboon (Papio cynocephalus), dogs, sheep, goats, rabbits, and mice (BALB/c, C57BL, and SJL) were used without further purification, after lysis of washed red cells and removal of stromata by centrifugation at 23,000 g. Fetal hemoglobins were isolated from stroma-free lysates of erythrocytes from a neonatal baboon and a human cord-blood sample by chromatography in columns of CM-Sephadex.\(^27\) Hemoglobin $F_{\text{iso}}$ was isolated from the lysed cells of a newborn macaque by DEAE-Sephadex chromatography.\(^27\) Purity and stability of the various hemoglobin preparations were determined by isoelectric focusing.

**PMB Derivatives**

Pure p-hydroxymercuribenzoate (PMB) derivatives of $\alpha$ and $\beta$ chains from a normal human adult, a macaque with Hbs $A'$ and $A''$, and a baboon were isolated by the method of Bucci and Fronticelli\(^28\) as modified by Gabuzda.\(^29\)

**Cyanogen Bromide Fragments**

A mixture of normal $\alpha$ and $\gamma$ hemoglobin chains, both with isoleucine in position 75, were isolated from fetal hemoglobin by CM-cellulose chromatography\(^30\) and subjected to cleavage by cyanogen bromide. The resultant fragments, $\gamma$ CB-1 (residues 1 through 55), $\gamma$ CB-2 (56 through 133), and $\gamma$ CB-3 (134 through 146), were isolated by gel filtration and recovered by lyophilization as described by Nute and Mahoney.\(^31\)

**Immunizations**

Strain SJL mice initially received intraperitoneal (i.p.) injections of 200 $\mu$g of human hemoglobin in complete Freund’s adjuvant. A second i.p. injection of 100 $\mu$g of the same antigen in incomplete Freund’s adjuvant was administered 2 wk later. At 7–10 days after the second injection, serum samples were tested for anti-Hb antibodies, using the assay described below. Animals showing the highest immune responses received an additional i.p. injection of 50 $\mu$g of Hb in saline 3–4 days before preparation of spleen cell suspensions.

**Cellular Fusions**

Cellular fusion was carried out essentially as described by Fazekas de St. Groth and Schneidegger\(^32\) using PEG-4000 (Baker Chemical Co., Phillipsburg, N.J.) as the fusing agent. On the day of fusion, a
single cell suspension of approximately $2 \times 10^6$ cells was prepared from the spleen of each mouse that exhibited a strong immune response. Four to seven separate fusions were performed with the cells from a single spleen, each involving $5 \times 10^5$ NS-1 murine myeloma cells and $3 \times 10^6$ monoclonal cells of splenic origin. After fusion, the cells were plated in HAT medium at approximately $6 \times 10^4$ spleen cells and $10^5$ NS-1 cells per well in Costar 96-well microtiter plates, using $6 \times 10^5$ mouse peritoneal macrophages per well as a feeder layer. After incubation at 37°C for 5 days, cultures were inspected and the medium replaced. Microscopic examinations and changes of medium followed at 48-hr intervals, until wells containing hybrid cells showed 30% confluence. At this time, the medium was again replaced; 48 hr later, at which time most wells were 100% confluent, culture supernates were screened for the presence of anti-Hb antibodies.

Screening for Antibody Activity

The antibody assay was essentially the same as that described for assay of viral coat protein antibodies. The test antigen (50 µl Hb at 20 µg/ml in PBS unless otherwise noted), was adsorbed to microtiter plates during incubation for 18 hr at 37°C. Unadsorbed antigen was then removed by aspiration and free sites in wells were blocked by incubation with 1% gelatin in PBS for an additional 2 hr. Culture supernatants (50 µl) were added to each well and incubated at 37°C for 45 min. After washing each well 3 times with 0.25% gelatin in PBS and adding $10^5$ CPM of $^{125}$I-labeled Staphylococcus aureus protein A (IPA) in 50 µl of 0.25% gelatin solution, plates were incubated at 37°C for an additional 45 min. Following 3 more washes with PBS, the plates were dried and assayed for IPA binding by autoradiography.

One of the antibodies (monoclonal 51-7) was an IgGI antibody that did not bind protein A. This monoclonal was detected because fusion 51 was assayed by an indirect antibody assay. Culture supernates were added first to the wells, as described above. Subsequently, a second antibody (the IgG fraction of rabbit anti-mouse immunoglobulin) was added and incubated for 45 min at 37°C. Labeled protein A was then added and the plates were processed as described above.

Cloning and Subcloning

Cells from wells containing antihemoglobin antibodies were mini-cloned by plating, in HT medium, 5-15 cells per well in 3 or 4 microtiter plates. Each well also received feeder cells (3-6 $\times 10^5$ macrophages or $10^5$ thymocytes per well). Upon attainment of sufficient cellular density, assays of antibody activity were conducted as described above.

All positive miniclones were recloned at 30-100 cells per microtiter plate, with $10^5$ mouse thymocyte feeder cells per plate, in standard medium (primary cloning). Plates were visually scored for true clones at 7 days. The selected clones were transferred to wells containing fresh macrophages or thymocytes, maintained until sufficient densities for assay were attained, and then screened for the presence of antibody. To ensure the isolation of stable antibody-producing lines of single cell origin, cloning was repeated (secondary cloning) until over 90% of wells with growth showed antibody production. Clones showing the most vigorous growth and the highest levels of antibody production were then selected and expanded as permanent lines prior to storage (by freezing) or tumor production.

Tumor Production

Since the antibody-producing cells were interstrain hybrids (BALB/c NS1 cells x SJL splenocytes), progeny from BALB/c x SJL matings were used for in vivo hybridoma culture. Animals were primed with pristane by i.p. injection of 0.5 ml 2 wk or more prior to i.p. injection of $5 \times 10^5$ culture cells in 1 ml culture medium. Large tumors appeared in 1-2 mo; animals were sacrificed, ascitic fluids were removed, and the cells collected by centrifugation. After resuspension in sterile saline, cells were reinjected into mice (at least $10^3$ cells/animal). Animal-passaged cells grew into tumors in 1-2 wk. Ascitic fluids were assayed for high levels of IgG by cellulose-acetate electrophoresis and positive samples were stored at -70°C until further purification.

Purification of Antibodies

Antibodies were isolated from cell-culture supernates or ascites fluid by affinity chromatography on Protein A-Sepharose CL-4B (Pharmacia, Piscataway, N.J.) as described by Ey et al. Elution was monitored at 280 nm; peaks were pooled and assayed for antibody by the antibody-binding assay described above. Antibody-containing fractions were dialyzed against PBS and stored at -20°C.

Determination of Antibody Class

The antibody class was established by two-dimensional immunodiffusion (Ouchterlony plates) using commercial antibodies against various mouse IgG subclasses. Anti-IgGI (Miles Laboratories Inc., Elkhart, Ind.), anti-IgG2a (Meloy Laboratories Inc., Springfield, Va.), anti-IgG2b (Meloy), and anti-IgG3 (Miles) were tested for reactions against authentic IgG standards before use.

Semiquantitative Reactions

In addition to monitoring antibody assays by autoradiography as described above, the $^{125}$I bound in each well was eluted (200 µl 2M NaOH at 37°C for 2 hr) and counted in a gamma counter (Nuclear-Chicago). Initially, binding curves were generated by titration of antigen (Hb), followed by application of constant amounts of antibody and $^{125}$IPA. With some antibodies this procedure gave high background values and nonspecific binding. These were eliminated by application of constant amounts of antigen (usually 1 µg/well), followed by reaction with increasing amounts of antibody (antibody titration) and a constant amount of $^{125}$IPA (10$^5$ cpm/well).

RESULTS

Isolation of Antihemoglobin-Producing Hybridomas

Thirty-seven fusion experiments were performed, and the supernates from approximately 2000 wells per experiment were screened for the presence of anti-Hb antibodies. Cells in wells showing negative or weak reactions were discarded. When strong positive reactions were obtained (Fig. 1A), the population of cells in the positive well was expanded and used for minicloning (Fig. 1B), primary and secondary cloning (Fig. 1C), and further studies. From the 37 experiments, 10 different hybrid clones were established and the cells were cryopreserved. Here we describe the characterization of six of these antibodies. The findings described here were obtained using antibodies purified from ascitic fluids by Protein A-Sepharose chromatography.
Table 1. Antiglobin Chain Monoclonal Antibodies

<table>
<thead>
<tr>
<th>Line</th>
<th>Specificity</th>
<th>Class</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-2</td>
<td>(\beta)</td>
<td>IgG2b</td>
</tr>
<tr>
<td>16-2</td>
<td>(\beta)</td>
<td>IgG2b</td>
</tr>
<tr>
<td>30-3</td>
<td>(\beta)</td>
<td>IgG2a</td>
</tr>
<tr>
<td>31-2</td>
<td>(\alpha, \beta)</td>
<td>IgG2b</td>
</tr>
<tr>
<td>37-8</td>
<td>(\beta)</td>
<td>IgG1</td>
</tr>
<tr>
<td>45-1</td>
<td>(\gamma, \beta)</td>
<td>IgG1</td>
</tr>
<tr>
<td>51-7</td>
<td>(\gamma)</td>
<td>IgG1</td>
</tr>
</tbody>
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Fig. 1. Autoradiography of antibody assay of cell culture supernates. HbA (1 \(\mu g/well\)) was adsorbed to all wells as described in Materials and Methods. Authentic anti-HbA antibody was added to the first well (upper left) as a positive control. All other wells received supernates from the wells in which the hybrid cells were grown. (A) Master fusion plate showing one well with anti-Hb-secreting hybrids. (B) Miniclones from cells in A. (C) Primary clones from miniclones.

Approach Used for Characterization

Characterization of the monoclonals was aimed at identifying the human globin-chain specificity, the non-human hemoglobins to which the antibodies bind, and if possible, the structural sites of the hemoglobin chains with which the antibody might react. Initial assignments of specificity were made by inspecting the results of Table 1.

Fig. 2. Binding of monoclonal 16-2 to various globins. (A) Human HbA (\(\bullet\)), human HbF (\(\circ\)). (B) Human PMB-\(\alpha\) chains (\(\triangle\)), human PMB-\(\beta\) chains (\(\bullet\)), and human Hb \(\gamma\) (\(\Delta\)). (C) Macaque HbA (\(\bullet\)), baboon HbA (\(\circ\)), and murine (SJL) HbA (\(\Delta\)). Preparation of hemoglobins and globin chains, purification of the monoclonal antibody, and the antibody assay procedures are described in text. Antibody binding was determined by autoradiography overnight, then bound IPA was eluted from each well and counted (see Materials and Methods).
CHAIN-SPECIFIC MONOCLONAL ANTIBODIES

Fig. 3. Binding of anti-adult 
Hb antibodies to various hemo-
globins. Relative binding is deter-
mained from the midpoints of 
binding curves, such as those shown in Figs. 2–7, using HbA as 
a standard.

of autoradiography of plates in which the antibodies 
were reacted with various human hemoglobins adsorbed 
to the wells. Subsequently, semiquantitative reactions 
were performed with human hemoglobins (HbA, A, and F) or hemoglobin chains (Hb γ and PMB-α, and 
PMB-β chains). The antibodies were further character-
ized by reaction with PMB-α and PMB-β chains from a 
baboon and macaque as well as several other animal 
hemoglobins. Specificities of the antibodies to human 
hemoglobins and antibody subclasses are shown in 
Table 1.

Monoclonal 16-2

The antibody produced by line 16-2 reacts with 
HbA, but the binding to HbF is insignificant (Fig. 
2A). The antibody binds to the human PMB-β sub-
units but, at the same antibody concentrations, there is 
evidently no binding to Hbγ or the PMB-α chain (Fig. 
2B). Monoclonal 16-2 thus appears to recognize an 
antigenic determinant that is present in the human β 
subunit but not in α or γ subunits.

Reactions carried out with adult Hbs from the 
baboon, macaque, and BALB/c mouse showed binding 
of the monoclonal with macaque HbA but not with the 
hemoglobins of the mouse or baboon (Fig. 2C). These 
findings suggested that the antibody has properties 
similar to the previously described antibody Hb β 
3-2. Additional binding studies with hemoglobins 
from a rabbit, goat, sheep, horse, and dog (Fig. 3) 
indicated specificities identical to those previously 
observed with Hb β 3-2. Additional reactions with hemoglobins 
Niteroi and Ocho Rios showed binding of the antibody 
to the former but absence of binding to the latter (data not shown). From these results we concluded that 
monoclonal Hb β 16-2 most likely recognizes the same 
antigenic determinant as monoclonal Hb β 3-2.

Monoclonal 37-8

Primary screening suggested that this monoclonal 
reacts with HbA but not HbF, and this was supported 
by the semiquantitative assay (Fig. 4A). The mono-
clonal binds to human PMB-β chains but not to Hb γ, 
or PMB-α chains (Fig. 4B).

Reactions of antibody 37-8 with baboon and 
macaque PMB-α and PMB-β chains (Fig. 4C) are 
virtually indistinguishable from its reactions with human 
hemoglobin chains (Fig. 4B); however, the antibody binds 
to the baboon HbA tetramer less efficiently than it 
binds to macaque or human HbA (Fig. 3). No binding 
was observed with adult Hb from rabbit, dog, sheep, or 
goat or any hemoglobins of murine origin (Fig. 3). These 
findings suggest that monoclonal 37-8 is specific 
for β subunits and that it is different from monoclonals 
Hbγ 3-2 and Hb β 16-2.

Monoclonal 30-3

The autoradiographic screening of the supernates 
from positive wells of hybrid cells suggested that this 
antibody is specific for human HbA and does not react 
with HbF. Semiquantitative reactions with antibody 
purified from the ascites fluid showed binding to HbA 
and weak binding to HbF.

Canine HbA and baboon HbA react with the anti-
body as efficiently as human HbA (Fig. 3). Macaque 
HbA shows weaker binding (cf., Fig. 5, A, B, C) while 
HbA from rabbit, horse, sheep, goat, and various 
murine strains are negative (Fig. 3).

An interesting characteristic of this antibody is that 
it binds more efficiently to HbA than to β-hemoglobin 
subunits (Fig. 5A). The same reaction pattern was 
noted when the binding of HbA and PMB-β subunits 
was compared using hemoglobin and globin chains 
from baboons and macaques (Fig. 5, B and C). It is
I. Antibody/well

Fig. 5. Binding of monoclonal 30-3 to primate HbA and α and β chains. (A) Human HbA (○), PMB-α chains (△), and PMB-β chains (●) and Hb γ4 (Δ). (B) Baboon PMB-α chains (○), Macaque PMB-β chains (●), baboon PMB-α chains (△), and Macaque PMB-α chains (●).

thus possible that this antibody recognizes an antigenic site that is of optimal conformation when the hemoglobin is in the tetrameric form.

Monoclonal 31-2

On screening, this antibody was found to react with both HbA and HbF. Subsequent semiquantitative studies showed that binding to HbF was about 80% of that to HbA (Fig. 6A). The antibody binds to human α and β subunits but does not react with Hb γ4 (Fig. 6B). Monoclonal 31-2 thus appears to recognize an antigenic determinant that is common to the α and β subunits and is designated as Hb αβ 31-2.

The antibody binds to the HbA of the baboon and macaque, though less efficiently than to human HbA.

Fig. 4. Binding of monoclonal 37-8 to various globins. (A) HbA (○), HbA2 (●), and HbF (Δ). (B) PMB-α chains (○), PMB-β chains (●), and Hb γ4 (Δ). (C) Baboon PMB-β chains (○), Macaque PMB-β chains (●), baboon PMB-α chains (△), and Macaque PMB-α chains (●).

Fig. 7C, similar to that of the HbA from the macaque and baboon.
HbF from the baboon shows weak binding. There is no binding of the monoclonal to fetal hemoglobin isolated from sheep. PMB-β chains from the baboon react with the monoclonal as efficiently as human β-chain (Fig. 9B). However, the monoclonal failed to react with HbA from the baboon (Fig. 9C); also, no binding was noted when HbA from macaque, dog, sheep, and BALB/c or C57BL mice were used in quantitative assays (Fig. 9C). There was excellent binding of the antibody to adult hemoglobin from the rabbit (Fig. 9C).

Monoclonal 51-7

The initial screening data from culture-cell supernates showed this antibody bound HbF but not HbA. Semiquantitative assays confirmed this observation (Fig. 10A), and additional reactions with globin chains showed the binding to be specific to γ-chains (Fig. 10B). Monoclonal 51-7 binds to baboon HbF and macaque HbF as well as it binds to human HbF, but does not react with sheep HbF (Fig. 10C). No binding was observed with rabbit, dog, sheep, or murine Hb (Fig. 11).

Reactions with cyanogen bromide γ fragments indicated that, like monoclonal 45-1, the antigenic site is located in fragment one (residues 1–55). However, the
of the seven monoclonals we have characterized, three (3-2, 16-2, 37-8) are \( \beta \)-specific. One (51-7) is \( \gamma \)-specific. One (45-1) recognizes a common antigenic determinant in \( \gamma \) and \( \beta \) subunits, one (31-2) recognizes a determinant common to the \( \alpha \) and \( \beta \) subunits, and one (30-3) recognizes an antigenic determinant that is expressed mainly in the \( \alpha \beta_2 \) tetramer. In distinguishing these monoclonals and identifying their specificities, the reactions with isolated hemoglobin subunits were essential. Without this step, monoclonal 45-1

![Diagram](https://via.placeholder.com/150)

**DISCUSSION**

Of the seven monoclonals we have characterized, three (3-2, 16-2, 37-8) are \( \beta \)-specific. One (51-7) is \( \gamma \)-specific. One (45-1) recognizes a common antigenic determinant in \( \gamma \) and \( \beta \) subunits, one (31-2) recognizes a determinant common to the \( \alpha \) and \( \beta \) subunits, and one (30-3) recognizes an antigenic determinant that is expressed mainly in the \( \alpha \beta_2 \) tetramer. In distinguishing these monoclonals and identifying their specificities, the reactions with isolated hemoglobin subunits were essential. Without this step, monoclonal 45-1
CHAIN-SPECIFIC MONOCLONAL ANTIBODIES

This anti-β-chain monoclonal binds to the macaque β-chain, but not to the baboon β-chain. By examining those sites in which both human and macaque β-chain differ from the β-chains of the baboon, we restricted the possible antigenic determinants to two sites, one in position β 43, the other in position β 52. We subsequently used mutant human hemoglobins to test this hypothesis. Hb Niteroi, a mutant from which the residues normally found in sites β 42 to β 44 have been deleted, reacted with the antibody in a fashion identical to that of HbA. On the other hand, Hb Ocho Rios, in which aspartic acid is substituted by alanine in position β 52, failed to bind the monoclonal. Reactions with hemoglobins of other species suggested that the antigenic determinant recognized by 3-2 extends from residue 51 to residue 55 of the β subunit. From the results of binding studies of monoclonal 16-2 to hemoglobins of the macaque and baboon as well as the two variants (Hb Ocho Rios and Niteroi), we concluded that monoclonal 16-2 recognizes the same antigenic site as 3-2. Similar deductions about the portions of the globin chains in which the antigenic determinants are located can be made about monoclonal 45-1.

Monoclonal 45-1 binds to HbF but not to HbA. However, the antibody recognizes β-chains. The absence of binding to HbA and presence of binding to β-chains suggests that an antigenic determinant of the

![Diagram of binding mononclonal 51-7 to various globins.](image)

would have been considered a HbF-specific reagent, even though it recognizes a determinant in the β subunit. The binding reactions with hemoglobins of various species were also essential to characterization of the monoclonals. They unequivocally showed that 16-2 is identical to the previously described 3-2, while 30-3 is distinguished from these two monoclonals by its ability to bind to HbA and the β-chain of the baboon.

Comparative binding reactions of antibodies with a series of homologous proteins of various species have been used to define the antigenic determinants of myoglobin and cytochrome-c. We first used this approach in the characterization of monoclonal 3-2.

![Diagram of binding of antifetal Hb antibodies to various hemoglobins.](image)
β subunit, which is “covered” in the αβ2 tetramer but not in the αγ2 tetramer, is recognized by the antibody. From the study of γ-chain cyanogen bromide fragments it appears that the determinant is located in the first 55 residues of the γ or β-chain. Since the monoclonal does not bind α subunits, the possible antigenic determinant would be located in a structural site in which γ and β subunits are alike, but are different from the α subunits. Also, the determinant occupies a structural site at which both human and baboon β subunits (showing similar reactions, Fig. 9B) differ from the β subunit of the macaque (Fig. 9B). Since rabbit HbA shows a positive reaction with monoclonal 45-1, the site would be identical in rabbit β and human β and γ sequences.* From such comparisons of primary structures we have preliminarily deduced that monoclonal 45-1 recognizes a sequence that lies between residues β12 and β20. Studies of human mutant hemoglobins with abnormalities located in these structural sites can serve to test this hypothesis.

All the monoclonals we have prepared fail to recognize adult murine Hb. Although hemoglobin is an intracellular protein, intravascular lysis or destruction of the senescent red cells in the reticuloendothelial system may expose the molecule to the immune system of the host and lead to its recognition as “self.” It thus appears that antibodies recognizing determinants in which the human molecule differs from murine hemoglobin are most likely expected to be produced with immunization of mice. There may be a limited number of such determinants.37 It is also of interest that the antigenic determinants identified through the studies of monoclonals 3-2 and 16-2 and the one proposed from 45-1 are located in regions of hemoglobin that are analogous to antigenic regions of myoglobin identified by the studies of Atassi and colleagues.37

The monoclonals described here provide useful tools in the investigation of expression of globin genes in various experimental systems. They have been applied in studies of hemoglobin expression in a human erythroleukemia line38 and in erythroid clones produced from human burst-forming units.39 The absence of cross-reaction with mouse globins will allow use of these monoclonals for testing expression of human β or γ globin genes in transformed mouse erythroleukemia cells. Monoclonals that recognize the globin chains of macaque and baboon can be applied in studies of γ and β globin expression in erythroid cultures produced by cloning erythroid progenitors from these species.

*Negative reactions (adult dog, sheep, goat, and mouse Hb and sheep HbF) are less informative, since reactions with PMB-β chains were not carried out.

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