Analyzing Glucose Phosphate Isomerase Isozymes in Chimeric Mouse Tissues by Electrophoresis

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**INTRODUCTION**

Mouse strains carry different alleles at the ubiquitously expressed Gpi1 (glucose phosphate isomerase) locus (Gpi1\(^a\), Gpi1\(^b\), Gpi1\(^c\)), and this is the basis for a widely used method for determining the genotypic composition of different tissues in mouse chimeras. To determine chimerism by this method, it is necessary to separate the differently charged isozymes from tissue homogenates electrophoretically and to visualize them using a color reaction. Because GPI is a dimer, tissues that normally form by cell fusion (e.g., skeletal muscle) have a heterodimeric form of GPI in a chimera.

**RELATED INFORMATION**

For the strain distribution of the three Gpi1 variants, see Lyon et al. (1996). For information on chimeras, see *Assembling Aggregates between Diploid Embryos* (Nagy et al. 2006a), *Assembling Aggregates between Embryonic Stem (ES) Cells and Diploid Embryos* (Nagy et al. 2006b), *Assembling Aggregates between Diploid and Tetraploid Embryos* (Nagy et al. 2006c), and *Assembling Aggregates between Embryonic Stem (ES) Cells and Tetraploid Embryos* (Nagy et al. 2006d). The genotypic composition of the chimeric tissues can be determined by comparing artificial mixtures of the two isozyme variants from tissues from the two different strains used either visually (±5%) or by densitometry (Behringer et al. 1984).

**MATERIALS**

**Reagents**

- Acetic acid (5%)
- Control blood sample

*Use equal volumes of blood from parental-strain mice homozygous for Gpi1\(^a\), Gpi1\(^b\), or Gpi1\(^c\). Dilute the samples 10X in 1X PBS and store them at ~20°C.*

- Embryo tissue samples
- Light paraffin oil (Fisher)

*Paraffin oil sold by pharmacists for human medicinal use may also be used. See Troubleshooting.*

- 1X phosphate-buffered saline (PBS)
- GPI stain, freshly prepared
- Tris-glycine gel buffer (pH 8.1)

**Equipment**
Gel box (custom-made [Helena Laboratories] or standard) with power supply

Glass plate

Ice

Lids of staining troughs (Raymond A. Lamb, E106)

Microscope slide marked with 10 equal divisions (see Step 3.iv)

Microscope slides

Permanent marker

Pipetman or hand-drawn Pasteur pipettes (see Step 4)

Tissue-culture dish (96 well)

Tissues or paper towels

Titan III cellulose acetate plates (60 x 76 mm; Helena Laboratories)

For the custom-made Helena gel box, three Titan III plates can be run simultaneously and, excluding two controls per plate, approximately eight samples can be run on each plate. For the standard horizontal gel boxes, the number of plates run simultaneously is determined by the width of the gel box.

Whatman 3 MM filter paper

METHOD

1. Place embryo tissue samples in 1X PBS or H2O in a 96-well tissue-culture dish. Place small fragments (e.g., the allantois from a 9.0-d embryo) in ~15 µL of 1X PBS and cover the drop with light paraffin oil to prevent evaporation. Intact 9.0-d embryos require at least 200 µL of 1X PBS. Freeze-thaw the tissue culture dish at least twice before running the samples.

2. Slowly immerse three Titan III cellulose acetate plates in a beaker of Tris-glycine gel buffer to ensure that no air bubbles are trapped. Soak them for 30 min before loading the samples.

3. Remove the first plate from the gel buffer, and prepare it as follows:
   i. Place the cellulose acetate surface down on a clean glass plate.
   ii. Wipe the plastic (shiny) surface of the plate dry with a tissue.
   iii. Draw a 60-mm line with a permanent marker pen across the plate, ~1 cm from the bottom.
   iv. Mark 10 equal divisions, leaving ~3 mm at either side.
   
   It is convenient to use as a template a microscope slide that already has the divisions marked on it.
   v. Number the divisions 1-10 beneath the line and label the plate at the bottom.
   vi. Turn the plate cellulose-acetate-surface-up and gently blot the bottom third with a tissue.
   vii. Place a strip of Whatman 3MM filter paper soaked in gel buffer over the opposite end to prevent drying out.

4. Using a Pipetman or a series of fine hand-drawn Pasteur pipettes (use a different tip or pipette for each sample), load ~1 µL of control sample containing both isozymes, onto divisions 1 and 10 of the plate. Load 1 µL of each sample onto each of the remaining eight divisions.
5. Place the plate into the gel box containing gel buffer with the plastic (shiny) surface uppermost and the cellulose acetate surfaces at either end resting on wicks (Whatman 3MM) immersed in the gel buffer. *Keep in mind that the samples will run from positive to negative.*
   i. To ensure even electrophoresis, apply weight to each plate with two parallel piles of about six microscope slides.
   ii. Place ice in the central portion of the gel box, under the plates but not touching them, to reduce heating effects and preserve enzyme activity (especially for \( \text{Gpi1}^c \), which is heat-labile).

6. Load the samples on the remaining plates and then run the gel at 200-250 V (4 mA) for 55-60 min. Reduce the running time for \( \text{Gpi1}^c \) because the enzyme is less stable (i.e., 30 min).

7. Stain the gel plates horizontally using freshly prepared GPI stain. *The lids of staining troughs are an ideal size for this.*
   i. Remove the plates and place cellulose acetate surface down, taking care to avoid air bubbles, in ~3 mL of GPI stain.
   ii. Place a light-tight cover over the plates while staining, but do not move the cover once the plates are placed in the stain because this will cause smearing.
   iii. Stain for 20-40 min, depending on the activity in the samples.

8. Rinse the plates in \( \text{H}_2\text{O} \) and fix for 5 min in 5% acetic acid.

9. Rinse twice in \( \text{H}_2\text{O} \).

10. Dry vertically.

**TROUBLESHOOTING**

**Problem:** Paraffin oil may be toxic.

**Solution:** Some investigators sterilize paraffin oil by autoclaving. However, others believe that this greatly increases toxicity and is not necessary because cultures containing antibiotics rarely become infected. In either case, the potential toxicity of batches of oil should be checked on spare embryos.

**REFERENCES**


Acetic acid (concentrated)

Acetic acid (concentrated) must be handled with great care. It may be harmful by inhalation, ingestion, or skin absorption. Wear appropriate gloves and goggles. Use in a chemical fume hood.

Recipe

GPI stain

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 mg/mL fructose-6-phosphate</td>
<td>1 mL</td>
</tr>
<tr>
<td>Glucose-6-phosphate dehydrogenase (Sigma G8289)</td>
<td>10 µL</td>
</tr>
<tr>
<td>0.2% (w/v) MgCl$_2$</td>
<td>9 mL</td>
</tr>
<tr>
<td>2.7 mg/mL NADP</td>
<td>1 mL</td>
</tr>
<tr>
<td>2.7 mg/mL 4-Nitro blue tetrazolium chloride (NBT)</td>
<td>1 mL</td>
</tr>
<tr>
<td>Tris-citrate buffer (pH 8.0)</td>
<td>1 mL</td>
</tr>
<tr>
<td>10 mg/mL pregnant mare serum (PMS)</td>
<td>30 µL</td>
</tr>
</tbody>
</table>

Freshly prepare the stain. Mix all reagents, adding PMS at the last minute.

Recipe

Phosphate-buffered saline (PBS)

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add (for 1X solution)</th>
<th>Final concentration (1X)</th>
<th>Amount to add (for 10X stock)</th>
<th>Final concentration (10X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>8 g</td>
<td>137 mM</td>
<td>80 g</td>
<td>1.37 M</td>
</tr>
<tr>
<td>KCl</td>
<td>0.2 g</td>
<td>2.7 mM</td>
<td>2 g</td>
<td>27 mM</td>
</tr>
<tr>
<td>Na$_2$HPO$_4$</td>
<td>1.44 g</td>
<td>10 mM</td>
<td>14.4 g</td>
<td>100 mM</td>
</tr>
<tr>
<td>KH$_2$PO$_4$</td>
<td>0.24 g</td>
<td>1.8 mM</td>
<td>2.4 g</td>
<td>18 mM</td>
</tr>
</tbody>
</table>

If necessary, PBS may be supplemented with the following:

- CaCl$_2$•2H$_2$O 0.133 g 1 mM 1.33 g 10 mM
- MgCl$_2$•6H$_2$O 0.10 g 0.5 mM 1.0 g 5 mM

PBS can be made as a 1X solution or as a 10X stock. To prepare 1 L of either 1X or 10X PBS, dissolve the reagents listed above in 800 mL of H$_2$O. Adjust the pH to 7.4 (or 7.2, if required) with HCl, and then add H$_2$O to
1 L. Dispense the solution into aliquots and sterilize them by autoclaving for 20 min at 15 psi (1.05 kg/cm²) on liquid cycle or by filter sterilization. Store PBS at room temperature.

**Recipe**

**Tris-glycine gel buffer (pH 8.1)**

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Amount to add</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>14.4 g</td>
</tr>
<tr>
<td>Tris</td>
<td>3 g</td>
</tr>
<tr>
<td>H₂O</td>
<td>to 1 L</td>
</tr>
</tbody>
</table>

Store at 4°C.