GPI ANALYSIS

1. Materials

Buffer : Supre-Heme buffer (Helena Laboratories 5802)
10 x 14.6 g Tris EDTA - boric acid pH 8.2-8.6: one sachet per liter demi

Gel : Titan III (Helena Laboratories 3024)
25 cellulose acetate plates 94 x 76 mm

Comb : Super Z applicator kit (Helena Laboratories 4093)
12 sample applicator set with two sample wells

Electrophoresis apparatus : Zip Zone Chamber (Helena Laboratories 1283)

Wicks : Disposable wicks for Zip Zone Chamber (Helena Laboratories 5081)

2. Staining solution

50 x stock solutions contain

			Sigma
-	G6PD (Glucose 6-Phosphate Dehydrogenase)	50 U/ml	G-6378
-	F6P (Fructose 6-Phosphate)	75 mg/ml	F-3627
-	NADP	10 mg/ml	N-3886
-	Phenazine MethoSO_4	1.8 mg/ml	P-9625
-	MTT	10 mg/ml	M-2128

3. Electrophoresis

- Put the small tissue parts in eppendorf tubes.
- Add 30 µl PBS to the tubes.
- Freeze the samples at -70 °C or grind them directly with an eppendorf potter.
- Tissues can be frozen and thawn 2 to 3 times, or can be used immediately.
- Thaw the samples at RT and centrifuge for 2 min at 14.000 rpm.
- Soak the cellulose acetate plate very slowly in the buffer for 10 min.
- Bring 8 µl of samples in the slots of the applicator.
- Take standards: 129/OLA = AA DBA/2 = AA
  C57B/6 = BB P = AA
  CBA = BB FVB = BB
- Place the soaked gel <u>once</u> between blue paper towels; the gel should not dry! Work quickly.
- Stamp the comb into the applicator twice; stamp the comb once on the gel; Two rows of 12 samples can be loaded on one gel.
- Place the gel on the electrophoresis system:
  . Apply it upside down on the paper rows (wicks).
  . Press gel on the paper rows to make sure that there is contact.

- Running conditions: 300 Volts
  3 mA/gel
  Run for 90 min
  Samples run from + to -

2. Staining

- Prepare staining stock solution:
  . 200 µl of each staining solution (except the enzyme).
  . Warm up to 55 °C just before use.
- Make a 1% agarose solution in bidest; allow to cool to 55 °C.
- Add 9 ml of agarose solution to the staining stock solution.
- Add 200 µl of G6DP (50x) and mix thoroughly.
- Pour the mix over the gelplate until the plate is completely covered; shut from light.
- Reaction takes place in a few minutes.
- Take a photograph immediately (bands vanish rapidly):
  . time 1/125 s
  . diaphragm 11 or 16