Paper

Cytogenet Genome Res 1975;14:465-469

Appendix 1

Stains for six enzymes potentially applicable to chromosomal assignment by cell hybridization

J.E.		Anderson
Y.-S.		Teng
E.R.		Giblett

King County Central Blood Bank, Seattle, Wash.

This is a preliminary report on staining methods used for detecting six enzymes after electrophoresis. We do not guarantee the specificity of all of these enzymes, although there is preliminary evidence that most of them are correct. These techniques may be suitable for isozyme differentiation in man-mouse hybrid cells for purposes of chromosomal assignment.

1. Adenylate deaminase (AMPDA) (AMP aminohydrolase, EC 3.5.4.6) a. Reaction scheme: 2-stage staining technique

AMPDA

IMP

(1) AMP

K⁺ ATP (2)

5' nucleotidase

added substances are underlined

Inosine N P

Hypoxanthine

MTT

Xanthine oxidase

PMS

. Formazan (dark blue)

Xanthine

Supported by NIH grant No. AM-09745 from the U.S. Public Health Service.

296 (466) Anderson et al. Staining methods

b. Stain:

First staining mixture: 40 mg AMP, 200 mg KCl, 10 mg ATP, 10 ml of 0.1 M tris-HCl, pH 7.8, and 10 ml of 2 % Ionagar. Pour over gel; after 3 h incubation at 37° C, remove agar overlay and apply second staining mixture: 1 mg 5'-nucleotidase, 10 µl xanthine oxidase, 10 µl nucleoside phosphorylase, 2.4 mg MTT, 2.4 mg PMS, 10 ml 0.1 M tris-HCl, pH 7.8, and 10 ml of 2 % Ionagar.

c. Electrophoretic pattern (phosphate buffer, pH 7.5):

AMPDA activity appears as a single broad blue band between hemoglobin (near origin) and adenosine deaminase, which is also stained on the gel.

2. PRPP synthetase or ribose phosphate pyrophosphokinase (RPPK) (ATP-D-ribose-5-phosphate pyrophosphotransferase, EC 2.7.6.1)

a. Reaction scheme:

RPPKR-5-P + ATP $\gg$ PRPP + AMP

ATP

AK

ATP ADP

Pyruvate PEP

LDH

♦ NAD (defluorescent) Lactate

b. Stain:

12.4 mg R-5-P Na₂, 15 mg ATP, 2.5 mg PEP, 3.5 mg NADH, 0.25 ml of 2 M KCl containing 0.5 M MgSO₄ < i, 50 μl myokinase, 50 μl PK, and 100 μl LDH in 5 ml of 0.1 M Tris-HCl, pH 7.8. Use filter paper overlying the first 10 cm of the gel; incubate 2 to 3 h and observe under UV light.

c. Electrophoretic pattern (phosphate buffer, pH 7.5):

RPPK activity appears under UV light as a single defluorescent zone about 5 cm anodal to the origin.

3. Uridine diphosphate kinase (UDPK) (ATP:nucleoside diphosphate phosphotransferase, EC 2.7.4.6)

Anderson et al. Staining methods

297 (467)

a. Reaction scheme:

UTP

UDPK

UDP

ADP

ATP

V PGK j/

1,3-DPGA 3-PGA

-NADH

G-3-PDH

•NAD (defluorescent)

G-3-P

b. Stain:

15 mg UTP, 4.9 mg ADP, 8.6 mg 3-PGA, 0.2 ml of 0.2 M KCl containing 0.05 M MgSO₄, 8 mg NADH, 25 μl PGK, and 50 μl G-3-PD in 10 ml of 0.1 M Tris-HCl, pH 7.8. Saturate filter paper and apply to gel; incubate at 37° C for 2 to 3 h and observe under UV light.

c. Electrophoretic pattern (histidine-citrate buffer, pH 7.0):

There are at least five UDPK isozymes, four anodal and one cathodal to the origin. Adenylate kinase (AK) is also stained and it may overlie one or more additional UDPK isozymes.

4. Transketolase or 1-glycoaldehydetransferase (TrKt) (D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate glycoaldehyde transferase, EC 2.2.1.1)

a. Reaction scheme:

PR I Epimerase R-5-P — Ru-5-P ► Xu-5-P

R-5-P

TrKt

Sed-7-P + G-3-P

TPI

DHAP

NADH

α -Gly-PD

NAD

α -Gly-P

Can start with xylulose-5-P, but it is very expensive.

b. Stain: 5 mg R-5-P, 0.5 mg RPI, 10 μ l epimerase, 40 μ l α -GPD/TPI (Sigma), 1 mg thiamine pyrophosphate, 0.2 ml of 1 M MgCl₂, and 4 mg NADH in 10 ml of 0.1 M glycylglycine buffer, pH 7.4. Use filter paper overlay, incubate 2 h at 37° C, and observe under UV light.

298 (468)

Anderson et al. Staining methods

c. Electrophoretic pattern (histidine-citrate buffer, pH 6.5): TrKt appears under UV light as a single defluorescent band migrating about 2 cm cathodal to the origin.

5. Transaldolase or dihydroxyacetone transferase (TrAl) (D-sedoheptulose-7-phosphate:D-glyceraldehyde-3-phosphate dihydroxyacetone transferase, EC 2.2.1.2)

a. Reaction scheme:

TrAl

Erythrose 4-P + F-6-P $\rightleftharpoons$ Sed-7-P + G-3-P

TPI

DHAP

c

■ NADH

α -Gly-PD

NAD α -Gly-P

b. Stain:

6.6 mg F-6-P, 3.3 mg Ery-4-P, 4 mg NADH, and 40 μ l α -GPD/TPI (Sigma) in 5 ml of 0.04 M tris-citrate buffer, pH 7.6. Saturate filter paper overlay, incubate at 37° C for 1 to 2 h, and observe under UV light.

c. Electrophoretic pattern (histidine-citrate buffer, pH 7.0):

TrAl appears under UV light as two major, fast-moving defluorescent bands, and a slower-moving, fainter band.

6. Uridine monophosphatase or pyrimidine 5' nucleotidase (UMPH) (5'-ribo-nucleotide phosphohydrolase, EC 3.1.3.5)

a. Reaction scheme: 2-stage staining technique

UMPH

(1) UMP $\rightarrow$ Uridine + Pi

Ascorbic acid NH₄⁺ molybdate

GSH

(2) dark blue complex

Pi detected by method of Fisher et al. (1974): A blue phosphate-molybdate complex appears at sites of enzyme activity.

b. Stain: First staining mixture: 20 mg UMP, 5 mg reduced glutathione, 2 ml of 0.5 M MgSO₄, 10 ml of 0.1 M tris-HCl, pH 7.8, and 10 ml of 2 % Ionagar. Pour onto gel and incubate for 2 h. Remove agar and cover gel with second staining mixture: 1 g ascorbic acid dissolved in 2.5 % ammonium molybdate in 4 N H₂SO₄ and 10 ml

Anderson et al. Staining methods 299 (469)