Giemsa-trypsin Banding

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National Institutes of Health

Reagents

Earle's Balanced Salt Solution (BSS)
Fetal Bovine Serum
Giemsa stain, original azure blend
Harleco 620G/75
Gurr's buffer tablets, pH 6.8
Biomedical Specialties, Santa Monica, CA
Trypsin solution 2.5%
Invitrogen Corp., Cat. 15240-062
Water, sterile

Preparation

Trypsin working solution

Trypsin solution, 2.5%	1.5 ml
Earle's BSS	50 ml

Fetal bovine serum solution

Fetal bovine serum	1 ml
Earle's BSS	50ml

Gurr's buffer solution, pH 6.8
Dissolve 1 tablet in 1 L sterile water

Giema Stain working solution

Giemsa stain	1 ml
Gurr's buffer solution, pH 6.8	50 ml

Procedure

1. Age the air-dried slide overnight in a dry oven at 55-60°C. Remove and bring to room temperature just prior to banding.

2. Grasp slide with forceps and dip in trypsin solution. Use a back-and–forth motion and expose to solution for 8-10 seconds. Time may have to be adjusted, depending on the degree of banding.

3. Quickly remove slide from trypsin and dip in fetal bovine serum solution. Move slide back-and-forth in the solution, or dip 5 times.

4. Pre-rinse the slide by dipping in a third jar containing Gurr's buffer, using the same technique as in step 3.

5. Place slide in Giemsa staining solution for 8-10 min.

6. Rinse slide in distilled water, using the same technique as in step 3. (If slide is left in water too long, the stain will fade).

7. Allow slide to air dry. Examine without mounting, using dry objectives; coverslip with Permount for examination with oil objective.