



HiPer® G-Banding Teaching Kit

Product Code: CCK043

1. About the Kit

G-banding is the most widely used banding method for chromosome analysis. It is also known as GTG banding (G bands produced with trypsin and Giemsa). Prepared and aged slides are treated with the enzyme trypsin and then stained with Giemsa. This produces a series of light and dark bands that allow positive identification of each chromosome. The dark bands are A-T rich, late replicating, heterochromatic regions of the chromosomes while the light bands are C-G rich, early replicating, euchromatic regions. The G-light bands are biologically more significant because they represent the active regions of the chromosomes while the G-dark bands contain relatively few active genes.

In addition to a distinct banding pattern, individual chromosomes are identified according to their size and centromere position.

One stained slide with metaphase spreads has been supplied in the kit for demonstration purpose. Users can generate more slides using HiPer™ Karyotyping Teaching Kit (CCK012).

4. Materials required but not provided in the kit

Unstained slides with metaphase spread
 Coplin jars (PW354)
 Forceps
 Serological pipettes: 5ml and 50ml (PW1193, PW1197)
 AutoHiPette™ automatic pipettor (LA692)
 Pipettes and tips
 Microscopy slides
 Distilled water
 Microscope with 100X oil immersion objective

5. Directions for use

Users are advised to review entire procedure before starting the assay

5.1 Preparation of 1% Fetal Bovine Serum

Note: 1% fetal bovine serum solution should be prepared just prior to use.

1. Thaw fetal bovine serum overnight at 2 - 8°C.
2. Add 500µl fetal bovine serum in 50ml distilled water.
3. Swirl gently to mix the contents properly.

Note: Do not swirl vigorously. This will cause foaming.

5.2 Preparation of 1X Giemsa Stain Solution

1. Add 47.5ml distilled water in a Coplin jar.
2. Add 2.5ml of Giemsa stain solution in water.
3. Mix well by pipetting up and down.

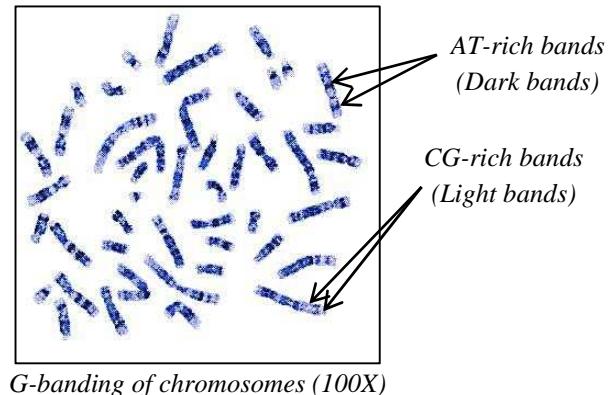
3. Kit Contents and Storage

Code	Description	Quantity	Storage
CCK043(A)	Trypsin Solution for G-banding	1 X 50ML	-30°C to -10°C
CCK043(B)	Giemsa Stain Solution 20X	1 X 5ML	15°C to 30°C
CCK043(C)	Fetal Bovine Serum	1 X 1ML	-30°C to -10°C
CCK043(D)	Gurr Buffer Solution	1 X 50ML	15°C to 30°C
CCK043(E)	Demonstration slide	1No.	15°C to 30°C

5.3 Procedure for G-banding

1. Age the air-dried metaphase slides overnight in a drying oven at 55°C to 60°C. Remove and bring the slides at room temperature.
2. Dispense 50ml trypsin solution, 50ml of 1% FBS solution, 50ml of 1X Giemsa stain solution and 50ml Gurr buffer in four Coplin jars. Label the jars as A, B, C and D.
Coplin jar A: Trypsin solution
Coplin jar B: 1% FBS solution
Coplin jar C: 1X Giemsa stain solution
Coplin jar D: Gurr buffer
3. Hold the slides with the help of forceps and immerse in a Coplin jar containing trypsin solution exactly for 8 - 10 seconds, moving the slide back and forth.
Note: The exposure time to trypsin should be adjusted depending on the quality of the cytogenetic specimen and the resulting banding.
4. Briefly rinse the slide in a Coplin jar containing 1% FBS to inactivate the trypsin.
5. Pre-rinse the slide by dipping in a Coplin jar containing Gurr buffer using the same agitation technique as in step 3.
6. Place the slide in a Coplin jar containing Giemsa stain solution for 8 to 10 minutes.
7. Rinse the slide in sterile distilled water until the stain no longer discolors the water using the same agitation technique as in step 3.
Note: Long exposure to water will result in de-staining of the slide.
8. Allow the slide to air dry. Examine by light microscopy using a phase contrast microscope to determine the quality of banding. Adjust trypsin exposure or duration of staining as required.
9. Once optimal banding quality has been achieved, analyze the slides. Store the slides in a slide box free of dust and dirt at room temperature.

6. Observations



G-banding of chromosomes (100X)

7. Storage and Shelf Life

On receipt, store the kit components at storage temperatures mentioned on individual product labels. Please refer individual product labels for shelf life.

Disclaimer:

User must ensure suitability of the product(s) in their application prior to use. Products conform solely to the information contained in this and other related HiMedia™ publications. The information contained in this publication is based on our research and development work and is to the best of our knowledge true and accurate. HiMedia™ Laboratories Pvt. Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are not intended for human or animal diagnostic or therapeutic use but for laboratory, research or further manufacturing use only, unless otherwise specified. Statements contained herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

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