Trypsin Solution for G-Banding

w/ 0.025% Trypsin in Dulbecco's Phosphate Buffered Saline

Product Code: TCL122

Product Description :

Trypsin
Molecular Weight: 23.4kDa
CAS No: 9002-07-07
EC No: 3.4.21.4

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.
TCL122 is 0.025% Trypsin in Dulbecco's Phosphate Buffered Saline which removes proteins from the chromosomes, in order for banding to occur

Activity:
One BAEE unit will produce aΔA253nm of 0.001 per minute with BAEE as substrate at pH 7.6 at 25°C in a reaction volume of 3.2ml (1cm light path).

One TAME unit hydrolyzes 1µmole of p-toluene-sulfonyl-L-arginine methyl ester (TAME) per minute at 25°C, pH 8.2, in the presence of 0.001M calcium ion.

One USP trypsin unit is the activity causing a change in absorbance of 0.003 per minute under the conditions specified.

Activity Conversion: 1 TAME unit = 19.2 USP or NF units = 57.5 BAEE Units

Directions for using Trypsin 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.

Quality Control:

Appearance

Colorless, clear solution.

pH

7.00 -7.60

Osmolality in mOsm/Kg H_2O

270-310

Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

Performance test

Light and dark bands were observed on metaphase chromosomes were treated with trypsin solution

Storage and Shelf Life:

Shelf life of the product is 24 months.
Upon receipt store the product at -20°C in a freezer that is not self-defrosting. Once thawed, the product is stable for about 2 weeks at 2-8°C.
Repeated freezing and thawing reduces enzymatic activity and should be avoided. Once thawed, the solution can be aliquoted in smaller volumes and frozen for future use.
Use before expiry date given on the product label.

Materials required but not provided

Gurr Buffer Solution pH 6.8 (TL1139)
Giemsa Stain Solution (TCL083)
Fetal Bovine Serum (RM10432)
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

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