CELL CYCLE PROTOCOL – BD CYCLE TEST PLUS DNA KIT

Materials

- The BD Cycletest Plus DNA kit contains the following (Cat.no 340242)
- Buffer Solution (3 vials, 50 ml per vial) Contains sodium citrate, sucrose, and dimethyl sulfoxide (DMSO) for the collection and/or freezing of cell suspensions.

- Solution A (10 ml)
Contains trypsin in a spermine tetrahydrochloride detergent buffer for the enzymatic disaggregation of the solid tissue fragments and digestion of cell membranes and cytoskeletons. Use Solution A at room temperature (20°C–25°C).

- Solution B (8 ml)
Contains trypsin inhibitor and ribonuclease A in citrate-stabilizing buffer with spermine tetrahydrochloride to inhibit the trypsin activity and to digest the RNA. Use Solution B at room temperature (20°C–25°C).

- Solution C (8 ml)
Contains PI and spermine tetrahydrochloride in citrate stabilizing buffer. The PI stoichiometrically binds to the DNA at a final concentration of at least 125 µg/ml. Protect Solution C from light and keep ice cold (2°C–8°C).

Consumables

- Powder-free gloves
- Falcon® disposable 17 x 100-mm capped polypropylene tubes, or equivalent
- Falcon disposable 12 x 75-mm capped polypropylene test tubes, or equivalent 50-µm nylon mesh or a Falcon disposable 12 x 75-mm tube with cell strainer cap 25-gauge x 1.5-inch
- hypodermic needles for tissue preparation 20-cc syringes
- Transfer pipets or disposable pipets 200-µl to 1000-µl adjustable micropipet and disposable tips - -
- Aluminum foil
- Disposable tissues

Equipment

- Vortex mixer
- Low-speed centrifuge (300 G) with swinging-bucket
- 12 x 75-mm sample tubes
- Vacuum aspirator with trap
For PI excitation, an argon-ion laser emitting at 488 nm and a filter detecting light at 580 nm–650 nm is optimal. See the appropriate cytometer user's guide for information.
- Ice bath

Staining

The staining procedure for DNA ploidy analysis requires a test sample of 5.0 x 10^5cells. Resuspend the cell in the Buffer solution

NOTE

Use Solution A and B at room temperature (20°C– 25°C). Keep Solution C ice cold (2°C–8°C) and protected from light.

1. Centrifuge the cell suspensions at 400 G for 5 minutes at room temperature (20°C–25°C).
2. Carefully decant all the supernatant, and tap off the last drop onto a tissue.
3. Add 250 µl of Solution A (trypsin buffer) to each tube and gently mix by tapping the tube by hand. Do not vortex.
4. Incubate for 10 minutes at room temperature (20°C– 25°C). Do not aspirate Solution A.
5. Add 200 µl of Solution B (trypsin inhibitor and RNase buffer) to each tube and gently mix by tapping the tube by hand. Do not vortex.
6. Incubate for 10 minutes at room temperature (20°C– 25°C). Do not aspirate Solution A and B.
7. Add 200 µl of cold (2°C–8°C) Solution C (PI stain solution) to each tube and gently mix by tapping the tube by hand. Do not vortex
8. Incubate for 10 minutes in the dark on ice or in the refrigerator (2°C–8°C).
9. Filter the sample through 50-µm nylon mesh into a labeled 12 x 75-mm tube, or use a 35-µm cell strainer cap and filter into a 12 x 75-mm tube.
The samples are now ready to be analyzed on the flow cytometer.
10. Cap or cover the prepared tubes and store at 2°C–8°C in the dark until flow cytometry analysis. For optimal results, acquire samples on the flow cytometer within 3 hours after the addition of Solution