Protocol

Observation of Fluorescent Proteins in Fixed Cells

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Fluorescent proteins (FPs) are popular reporters available for gene expression detection and determination of cellular identities in the mouse. This protocol can be used to detect green fluorescent protein spectral variants and proteins labeled with the fusion tag dsRed in fixed cells.

MATERIALS

It is essential that you consult the appropriate Material Safety Data Sheets and your institution's Environmental Health and Safety Office for proper handling of equipment and hazardous material used in this protocol.

Reagents

Cell culture
Mounting medium (Molecular Probes Inc., ProLong Antifade Kit, P-7481)
Paraformaldehyde (pH 7.4, 2% in PBS)

Equipment

Coverslips, prepared sterile (coating might be necessary for cell attachment)
Microscope equipped with FP visualization
Microscope slides
Whatman filter paper

METHOD

1. Place a coverslip onto a tissue culture plate, and plate the cells on the coverslip.
2. Grow the cells for desired confluence or length of time.
3. Fix the cells in 2% paraformaldehyde in PBS (pH 7.4) for 15 min at room temperature.
Do not fix in methanol or acetic acid because organic solvents destroy the light emission of some FPs.
4. Rinse the cells in 4 mL of PBS (pH 7.4), and then quickly wash the coverslips in 2 mL of PBS (pH 7.4) twice for 5 min at room temperature.
5. Place 10 μ L of mounting medium onto the middle of a glass microscopy slide.
6. Blot the edges of the coverslip gently against a piece of filter paper to remove excessive PBS.

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7. Place the coverslip with the attached cells facing down onto the mounting medium.
8. Observe the fluorescence.

RELATED INFORMATION

For additional advice, see Protocol: **Observation of Fluorescent Proteins in Living Cells** (Behringer et al. 2019).

REFERENCES

Behringer R, Gertsenstein M, Vintersten Nagy K, Nagy A. 2019. Observation of fluorescent proteins in living cells. *Cold Spring Harb Protoc* doi: 10.1101/pdb.prot092767.

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