

Polyethylene Glycol 1500 (PEG 1500)

Solution, sterile

Cat. No. 783 641

10 × 4 ml

Version 3, Dec. 2002

Store at 2-8°C

Product overview

Formulation	50% PEG 1500 (w/v) in 75 mM Hepes, pH 8.0, bottled under nitrogen; sterile (sterile-filtered, not autoclaved); ready for use.
Formula	HO (CH ₂ CH ₂ O) _n H.
Biological activity	PEG 1500 is biologically evaluated for high fusion efficiency.
Molecular weight	1500.
Typical analysis	Peroxides and aldehydes not detectable; free from Ca ²⁺ .
Application	Cell fusion induced by polyethylene glycol (PEG) has become a standard method in somatic cell genetics (1-4). PEG promoted cell fusion is also the standard procedure for the production of hybridoma cells (5-12).
Storage/Stability	Stable at 2-8°C until the expiration date printed on the label. Note: Store protected from light!

Working instruction for fusion of myeloma cells and mouse spleen cells

Protocol

Please refer to the following table:

Step	Action
1	Mix 10 ⁸ spleen cells (in 25 ml) and 2 × 10 ⁷ myeloma cells (in 25 ml) in serum-free medium in a conical tube.
2	Spin the cells down (5-10 min, 200 - 400 × g).
3	Remove the supernatant with a pasteur pipette. Note: Complete removal of the supernatant is essential to avoid dilution of PEG.
4	<ul style="list-style-type: none">Broke the pellet by gently tapping the bottom of the tube.Place the tube in a 37°C water bath and kept there during the fusion.
5	Add 1 ml 50% PEG 1500 pre-warmed to 37°C to the pellet using a 1 ml pipette, over a period of 1 min, continually stirring the cells with the pipette tip.
6	Continue stirring the cells in 50% PEG 1500 for further 1-2 min.
7	Add 1 ml medium pre-warmed to 37°C to the fusion mixture, continuously stirring as before, over a period of 1 min.
8	Add 3 ml medium pre-warmed to 37°C over a period of 3 min, continuously stirring the cells.
9	Add slowly 10 ml medium pre-warmed to 37°C.
10	Incubate for 5 min at 37°C.
11	Spin the cells down.
12	Discard the supernatant and resuspend the pellet in selection medium, e. g. RPMI 1640, 10% FCS (fetal calf serum) (v/v), non essential amino acids (1 ×), 2 mM glutamine, 1 mM sodium pyruvate, HAT-media supplement* (1 ×), 10% BM Condimed ¹⁾ H1 * (v/v) or 50-100 U/ml IL-6*.
13	Seed cells as usual.

References

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