Nupherin™

Catalog #: BML-SE225

ACTIVITY: The Nupherin™ reagent is a specially designed peptide formulation that serves as an enhancer of plasmid transfer and expression in lipofected cell lines. The Nupherin™ reagent is used with cationic lipid and is compatible with most commercially available cationic lipid formulations.

Use at 5 to 50µg Nupherin™ reagent per 1µg of plasmid.

STORAGE: Nupherin™ is supplied in sterile DNAse-free distilled water, frozen at 3 mg/ml.

Store frozen at –80°C.

On first use, aliquot the reagent and store at -80°C.

Thawed aliquots of Nupherin™ can be refrozen after use for long term storage or refrigerated for up to 1 week. Do not dilute the reagent.

Avoid repeated freeze/thaw cycles.

REFERENCES:

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7. R. Nashmi, et al. J Neurosci. 2003 23 11554
8. E.M. Slimko, et al. J Neurosci Methods 2003 124 75
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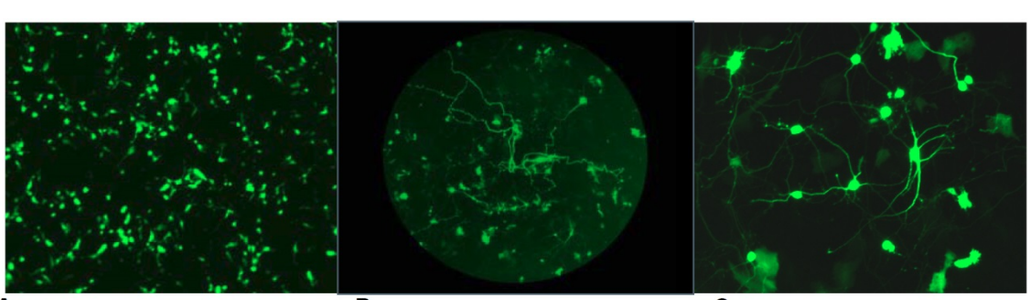

A. B. C.

Figure A: Chicken Embryonic Retinal Ganglia
Figure B. Primary neuron lipofection
Figure C. Embryonic rat primary hippocampal neuron

FOR RESEARCH USE ONLY.
NOT FOR USE IN DIAGNOSTIC PROCEDURES.

SAMPLE PROTOCOL FOR NUPHERIN™

1. Culture cells on glass or plastic coverslips placed in 6-well (9.6cm^2) or 24-well (2.0cm^2) plates or culture cells directly in 6-well or 24-well plates. Cells and cell lines can be transfected at subconfluence, confluence, or in a post-mitotic state.
2. TUBE A: For each well of a 24-well plate to be transfected, use 0.5 to 3µg plasmid DNA per well. 1µg or less of DNA is preferred and may result in higher transfection rates. Dilute plasmid into 150µl of serum-free phenol red-free DMEM or OPTIMEM™ in Tube A. Add 3 to 20µl of the Nupherin™ reagent (9 to 60µg) per µg plasmid to Tube A. Gently mix by pipetting (do not vortex). Incubate 15 min at room temperature before mixing with Tube B.
3. TUBE B: Add cationic lipid (e.g. 1 to 4µl Lipofectamine™ at 2µg/µl) to a final volume of 150µl serum-free phenol red-free DMEM or OPTIMEM™ in Tube B. For other cationic lipid reagents, use: 0.5-3µl Fugene™,1-3µl lipofectin™,1-4µl lipofectamine™, 2-6µl Geneporter™.
4. Gently mix Tube A and Tube B by pipetting (do not vortex). Incubate for 40 minutes at room temperature.
5. Rinse cells in serum-free medium, overlay cells in each 2-cm^2 well with the transfection solution (about 300µl).
6. Centrifuge the plate at 100g (1000 rpm in swinging bucket rotor) for 3 to 5 min at room temperature.
7. Incubate the plate at 37°C for 0.5 to 4 hr. Replace with serum-containing growth medium.

APPLICATION EXAMPLE

Recommended Protocol for Post-mitotic Primary Neurons

	2.0cm^2 well (24-well plate)	**9.6cm^2 well (6-well plate)**
plasmid (1µg/µl)	1µl	2µl
Nupherin (3µg/µl)	10µl	20µl
OPTIMEM™(1X)	140µl	380µl
	150µl Tube A	400µl Tube A
Lipofectamine™ (2µg/µl)	1µl	3µl
OPTIMEM™ (1X)	150µl	400µl
	150µl Tube B	400µl Tube B

Gently overlay 300µl of transfection mixture (Tube A + Tube B) over 2.0cm^2-well or 800µl over 9.6 cm^2-well, centrifuge 100g for 3 min, incubate 30 min, and replace with growth media.

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