



Title: *Preparation of MAA-PEI*

No: RTLP-CONJ-1

Location:
Old CCRC Tripp Lab

Approval Date:
10 September 2004

Supersedes Date:

Materials:

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•Lab coat	•Reductacryl: DTT- conjugated to poly- styrene beads	•Collection tubes	•Pipettes
•Gloves	•DMSO	•dH ₂ O	•Pipetteman
•DNA plasmid to be tested	•0.6% Agarose Gel	•110°C oil bath	•Pipette Aid
•N-succinimidyl 3-(2- pyridyldithio) propionate (SPDP)	•Phosphate Buffered Saline (PBS)	•10mL round- bottomed flask	•Pipetteman tips
•polyethylenimine (PEI)	•NAP-10 columns	•Bovine or mouse serum albumen (BSA or MSA)	•Concentrated HCL
	•0.10 M NaHCO ₃ , pH 8.0-8.5		•pH meter
			•Stir plate
			•Hot plate
			•-80°C storage

Procedure:

Preparation of MAA-PEI mix:

1. SPDP/HSA solution:

- Dilute 1 mg SPDP in 40 µl DMSO.
- Dilute 50 mg MSA in 1 ml 0.10 M NaHCO₃, pH 8.0 – 8.5
- Add SPDP to MSA solution, dropwise, while MSA is stirring.
- Stir several hours (4 hr, room temperature)
- Put mix on NAP-10 column equilibrated in PBS
- Dilute effluent to 3.5 ml in PBS

2. PEI solution:

- Adjust the pH of 72.5 mg of a 50% (w/v, will actually be 36 mg of PEI) PEI in 1 ml water to 8.0 – 8.5 with concentrated HCl. (if pH is overrun, that's ok, just bring back down; use concentrated HCl to adjust, 10 ul at a time)
- Add 0.25 mg of SPDP in 10 µl DMSO to PEI solution, dropwise, while PEI is stirring
- Stir several hours (4 hr, at room temperature)
- Adjust pH to 7.0 – 7.5

- Add 20 mg of Reductacryl (will work ok at pH between 4 – 8)
- Stir 30 mins at room temperature
- Put mix on NAP-10 column equilibrated in PBS (reductacryl beads will not hurt column)
- PEI is eluted

*****Notes on NAP-10 columns:** *to use NAP-10 columns, first wash in 3 column volumes of PBS. Load a total of 1 ml sample onto the column and let flow through. Then add 1.5 ml of PBS and collect this 1.5 ml of flow-through – will contain MSA or PEI solution of interest.*

3. Combine solutions:

- Add PEI solution to MSA solution all at once (final volume 5 ml; may see some particles here, ok so long as they are few and small)
- Stir overnight at room temperature (albumin now at 10 mg/ml...VERY IMPORTANT)
- Adjust pH to 5.5 – 6.0 with HCl
- Heat conjugate with stirring in 10 ml round-bottomed flask in an oil bath set at 110°C for 15 min (intraflask temperature approx 80° – 90° C) – solution should be milky
 - Note: use hot plate at setting 2-3, heat and stir vigorously in beaker – instead of oil bath
- Cool flask under running water
- Adjust pH to 7.0 – 7.5 with 2M NaOH
- Adjust volume to 5 ml

** aggregated conjugates may be frozen at –80°C with no loss of activity. Can also undergo numerous freeze-thaw cycles*

4. Determine actual concentration of PEI N available for DNA binding:

- Dilute particle suspension 1:100 in PBS
- To an aliquot of diluted particle suspension add 1 µg plasmid in 5 µl PBS, while gently vortexing. (use a range of aliquots of diluted suspension, from 2 – 10 µl)
- Incubate at room temperature 20 mins
- Run 4 µl aliquot of each sample out on gel (0.6% agarose in TBE)

** the lowest volume lane where no plasmid enters the gel represents a ratio of PEI N to DNA P of 2:1. This is where all negative charges are neutralized. The concentration of PEI N in the sample can be calculated as:*

$$[\text{PEI N, } \mu\text{mol}/\mu\text{l (M)}] = (100 \times 2 \times 0.003) / \text{volume added to lane}$$

where: 100 = initial dilution factor

2 = 2:1 ratio for charge neutralization

0.003 = μmol of P/ μg of DNA (DNA contains 0.003 μmol P per μg)

An ideal N/P ratio is 15 +/- 3-4

5. Mix MAA-PEI and plasmids for *in vivo* transfection:

- Match desired amount of DNA with appropriate volume of MAA-PEI to achieve N/P ratio of 15:1.
- Dilute particles in PBS to desired volume (for IV injection in mouse, 1 μl or less per 175 μl PBS for 1 μg of DNA).
- Add plasmid dropwise (1 μg diluted in 25 μl PBS) with gentle vortexing
- Incubate suspension at RT for 20 – 60 mins before IV administration

**caveats: high-speed vortexing interferes with effective formation of transfecting particles, excess DNA (N/P ratio </- 10) reduces transfection efficiency, may cause particle clumping that blocks transfection*

** most reliable to prepare each dose individually rather than making a large batch that is given in aliquots to each mouse*