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We are still developing and optimizing this protocol

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Treatment of DNA with cisplatin

Martin O Pollard[1]

[1]Wellcome Sanger Institute

Martin O Pollard
Wellcome Sanger Institute

ABSTRACT

A protocol for treating Promega Human DNA with Cisplatin.

GUIDELINES

Dispose of excess cisplatin safely according to guidance received in SDS.

MATERIALS

25mg cis-Diammineplatinum(II) dichloride - Sigma Aldrich Product Number P4394
0.9% NaCl solution
Promega Human DNA - Human Mixture
Buffer
Gibson P20 pipette
Wide bore tips
a 1.8 mL Eppendorf tube .

SAFETY WARNINGS

Cisplatin safety warnings:
H300 Fatal if swallowed.
H315 Causes skin irritation.
H317 May cause an allergic skin reaction.
H319 Causes serious eye irritation.
H334 May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335 May cause respiratory irritation.
H350 May cause cancer.
Measure out in fume cupboard or enclosed chemical preparation unit; add solvent in Biosafety cabinet

Preparation of Cisplatin liquid

1

Safety information

Perform this step in a fume hood or use an enclosed scale to minimise exposure.
It is preferable to purchase cisplatin in pre-weighed out quantity where possible.

Weigh out 25 mg of cisplatin into 1000 mL of 0.9% NaCl and mix thoroughly to make a stock solution.

Store stock at 2-8 °C and protected from light to avoid precipitation.

2 Dilute 1 mL of your stock cisplatin solution into 99 mL of 0.9% NaCl and mix thoroughly.

Store diluted cisplatin at 2-8 °C and protected from light to avoid precipitation.

Treatment of DNA with Cisplatin

1h

3 Pipette 8.811 µL of Sample from stock tube at [M] 227 µg/ml to a 1.8 mL Eppendorf tube to achieve 2 µg of DNA.

4

Safety information

Perform this step in a fume hood

Add 7.27 µL of diluted cisplatin at [M] 0.83 micromolar (µM) to the tube

5 Incubate for 16:00:00 in the dark at 37 °C .

16h

6 Perform a SPRI bead clean-up (as previously described) to remove lingering cisplatin from supernatant.

6.1 Add 0 µL of [M] 0 Mass Percent SPRI beads to tube and 20% polyethylene glycol (PEG), 2.5 M NaCl buffer.

6.2 Attach tube to magnet and remove supernatant.

6.3 Resuspend DNA in 40 µL of buffer to achieve concentration of [M] 50 ng/µl