Methyltransferase Assay

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Abstract

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Guidelines

Reaction Conditions and Buffer, Materials:

- Reaction conditions: 50 mM Tris-HCl, pH 7.8, 10 mM EDTA, 0.5 mM DTT, 2 µCi Adenosyl-L-Methionine, S-Methyl (^{3}H), 3 µg DNA

- Preparation of reaction buffer, 10X

  3000.0 µL of 1 M Tris-HCl, pH 7.8

  1200.0 µL of 500 mM EDTA, pH 8.0

  1500.0 µL of d-H_2O

  To 95.0 µL of the reaction buffer, add 5.0 µL of 100 mM DTT.

  Use 5.0 µL per 50.0 µL reaction.

- Proteinase K, 2 mg/mL (autodigested at 37°C for 60 min)

- ss salmon sperm DNA, 5 mg/mL

- 20% TCA

- 5% TCA

- 500 mM NaOH

Protocol

Step 1.
Set up the assays, to contain 50.0 µL total per assay with 3 µg of DNA and 2 µCi of Adenosyl-L-Methionine, S-Methyl (^{3}H), and the enzyme to be tested.

Step 2.
Incubate the assays at 37°C for 2 hours.

DURATION
02:00:00

Step 3.
Add 30 µL (60 µg) of proteinase K to each assay.

Step 4.
Incubate at 65°C for 20 min.

DURATION
00:20:00

Step 5.
Add 4 µL (20 µg) of ss salmon sperm DNA as a carrier.

Step 6.
Add an equal volume (84 µL) of 20% TCA and incubate at 4°C for 10 min to precipitate the samples.

DURATION
00:10:00

Step 7.
Centrifuge the assays in the microfuge for 5 min at 4°C.

DURATION
00:05:00

Step 8.
Discard the supernatants.

Step 9.
Wash the pellets 2X with 200 µL of 5% TCA in the microfuge for 5 min at 4°C.

DURATION
00:05:00

Step 10.
Resuspend the pellets with 500 µL each of 500 mM NaOH.

Step 11.
Incubate the assays at 65°C for 20 min.

DURATION
00:20:00

Step 12.
Precipitate the samples with 500 µL of 20% TCA for 10 min at 4°C.

DURATION
00:10:00

Step 13.

Centrifuge the samples in the microfuge for 5 min at 4°C.

DURATION

00:05:00

Step 14.

Wash the pellets 2X with 200 µL of 5% TCA in the microfuge for 5 min at 4°C.

DURATION

00:05:00

Step 15.

Resuspend the pellet with 500 µL of 5% TCA.

Step 16.

Incubate in boiling water for 30 min (loosen the caps or puncture the caps of the tubes).

DURATION

00:30:00

Step 17.

Centrifuge the samples in the microfuge for 5 min at 4°C to remove the insoluble material.

DURATION

00:05:00

Step 18.

Count the supernatants in 10 mL of triton-toluene counting solution.