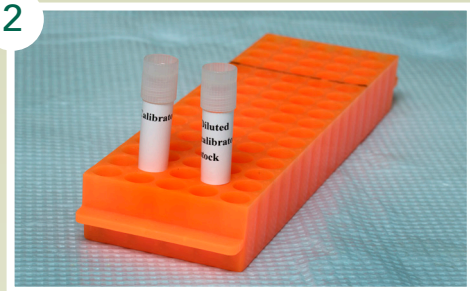
Part 4: Dilute Calibrator and Add to Calibrator Plate

1

Prepare calibrator working solutions about 15 min before use. The preparation is done on a different bench to prevent folate contamination.

2



Thaw 1 vial of 5-methyl-THF calibrator stock solution (1 µmol/L) for about 15 min at room temperature and mix well. To prepare a 1:5 diluted stock solution (200 nmol/L), add 400 µL of 0.5% sodium ascorbate to a 2-mL cryovial, then add 100 µL of calibrator stock solution and mix well.

3

Add approximately 45 mL of 0.5% sodium ascorbate each to two 50-mL grade A volumetric flasks labeled as Level I and Level II.

4

Transfer 50 µL of diluted stock solution to the Level I flask (1:1000 dilution; 200 pmol/L) and 250 µL to the Level II flask (1:200 dilution; 1 nmol/L).

5

Using a plastic transfer pipette with a fine tip, make up with 0.5% sodium ascorbate to the 50-mL volume mark. Be sure the bottom of the fluid meniscus touches the calibration line at eye level. This is a very critical step.

6

Slowly invert the volumetric flasks 20 times to thoroughly mix the calibrator working solutions. Move back to the sample preparation area. Change gloves to prevent accidental folate contamination.

7

Pour the Level I and Level II working solutions into two labeled basins.

8

Calibrator plate

Add level I and II working solution

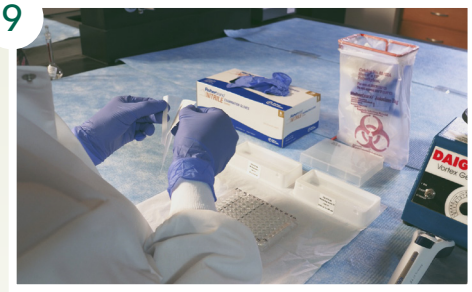
	1	2	3	4	5	6	7	8	9	10	11	12
A	0	0	25	50	75	100	30	40	50	60	80	100
B	0	0	25	50	75	100	30	40	50	60	80	100
C	0	0	25	50	75	100	30	40	50	60	80	100
D	0	0	25	50	75	100	30	40	50	60	80	100
E	0	0	25	50	75	100	30	40	50	60	80	100
F	0	0	25	50	75	100	30	40	50	60	80	100
G	0	0	25	50	75	100	30	40	50	60	80	100
H	0	0	25	50	75	100	30	40	50	60	80	100

Level I (µL) | Level II (µL)

Calibrator concentration in well (nmol/L)										
Blank	0.00	0.05	0.10	0.15	0.20	0.30	0.40	0.50	0.60	0.80	1.00

Add calibrator working solutions to plate #1 using the smaller 12-channel electronic pipette (20-300 µL) with the single pipetting function and 8 tips. Transfer appropriate volumes as shown here.

9



Gently hand-seal the calibrator plate with a sealing membrane and set aside. Change gloves to prevent accidental folate contamination of the sample plates in the next step.

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www.cdc.gov/nceh/dls/nbb.html

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CS299059-B