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

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.

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.

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).

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.

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.

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

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.

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.