5-Carboxylcytosine DNA Standard

Catalog No: 55014

Format: 0.5 µg

Quality Control: 5-Carboxylcytosine DNA Standard Kit is quality control tested by dot blot using Active Motif's 5-Carboxylcytosine antibody (Catalog No. 61225) at a 1:2,000 dilution with an overnight incubation. The 5-Carboxylcytosine DNA standard is tested alongside the equivalent Unmodified DNA standard. The 5-Carboxylcytosine DNA standard is specifically recognized by the antibody and there is no cross-reactivity for other DNA modifications or the Unmodified standard (Figure 1).

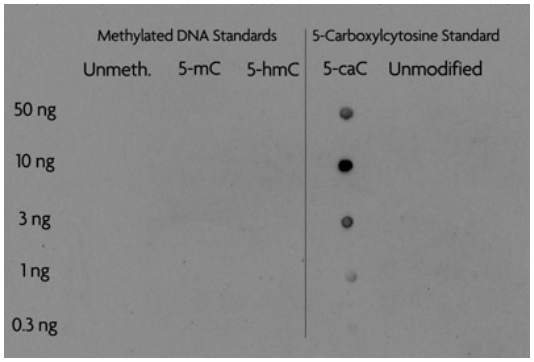

Figure 1:
Varying amounts of dsDNA from the Methylated DNA Standard Kit (Catalog No. 55008) or 5-Carboxylcytosine DNA Standard Kit were spotted onto a nylon membrane and probed with 5-Carboxylcytosine antibody (Catalog No. 61225, 1:2000 dilution).

Contents:
5-Carboxylcytosine DNA standard, 0.5 µg (50 ng/µl)
Unmodified DNA standard, 0.5 µg (50 ng/µl)

The 5-Carboxylcytosine DNA standard consists of a 38 bp double-stranded DNA oligonucleotide. The forward strand contains 8 carboxylcytosine DNA modifications, while the reverse strand contains 4 carboxylcytosine DNA modifications. The annealed dsDNA therefore contains a total of 12 carboxylcytosine residues. The Unmodified DNA standard is the equivalent dsDNA sequence without any cytosine modifications and can be used as a negative control.

Forward Strand: 5' -AGCCXGXGCXGXGCXGGTXGAGXGGCXGCTCCCGCAGC- 3'
Reverse Strand: 3' -TCGGGCGCGGCGXGGXCAGXTCGXCGGCGAGGGCGTCG- 5'

X indicates a carboxylcytosine residue in the 5-Carboxylcytosine standard
X indicates a cytosine residue in the Unmodified DNA standard

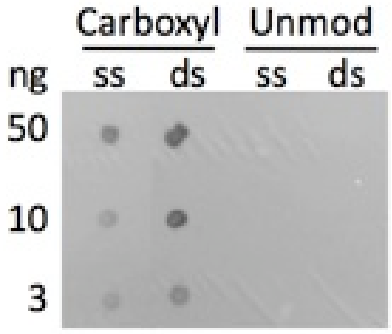

Figure 2:
Comparison of single-stranded DNA (ss) and double-stranded DNA (ds) from the 5-Carboxylcytosine DNA Standard Kit by dot blot with a 1:2000 dilution of 5-Carboxylcytosine Ab (Cat. No. 61225).

Dot Blot Protocol

1. Prepare the necessary dilutions of the 5-Carboxylcytosine DNA standards. We recommend between 3 ng - 50 ng.
2. For detection of ssDNA, denature the DNA standards with heat, 95°C for 10 minutes, immediately followed by transferring the denatured DNA standards to ice.
3. Spot 1 µl of each DNA dilution onto a positively charged nylon membrane. Allow the membrane to dry 5-10 minutes.
4. Block the membrane with TBST (10 mM Tris, 150 mM NaCl, 0.1% Tween-20) and 5% non-fat milk powder for 1 hour with rotation.
5. Dilute 5-Carboxylcytosine pAb (Catalog No. 61225) 1:2000 in TBST + 5% non-fat milk powder. Ensure that the entire membrane is covered with the antibody solution.
6. Incubate overnight at 4°C.
7. Wash the membrane 3 times with TBST, 10 minutes per wash.

Quality Control:

Dot Blot Protocol continued

8. Incubate with HRP-conjugated anti-rabbit IgG secondary antibody diluted 1:2000 in TBST + 5% non-fat milk powder for 1 hour at room temperature.

9. Wash the membrane 3 times with TBST, 10 minutes per wash.

10. Develop with ECL reagents according to manufacturer's instructions. Optimize exposure time as needed.

Storage and Guarantee: DNA standards should be stored at -20°C. This product is guaranteed stable for 6 months from date of receipt when stored properly.