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EasyMag DNA Methylation Kit

Catalog Number: D201-1, D201-2

Table 1. Kit Components and Storage

Kit Component	D201-1 (96 preps)	D201-2 (4×96 preps)	Storage	Stability
CT Conversion Reagent*	1 bottle	4 bottles	Room Temperature	The product is stable for one year when stored as directed.
Dilution Buffer	3.5 mL	14 mL		
Dissolving Buffer	600 µL	2×1.2 mL		
Binding Buffer	60 mL	2×120 mL		
Wash Buffer**	15 mL	2×25 mL		
Desulphonation Buffer	20 mL	80 mL		
Elution Buffer	2×1.5 mL	15 mL		
MagPure Beads	2 mL	8 mL		

***** 9 mL water, 3 mL Dilution Buffer, and 500 µL Dissolving Buffer must be added per tube of **CT Conversion Reagent** prior to use.

****** Add 60 mL of 100% ethanol to the 15 mL **Wash Buffer** concentrate (D201-1) or 100 mL of 100% ethanol to the 25 mL **Wash Buffer** concentrate (D201-2) before use.

Product Description

EasyMag DNA Methylation Kit integrates DNA denaturation and bisulfite conversion processes into one-step coupled to a magnetic bead based clean-up for high-throughput methylation analysis. Also, the kit has been streamlined for high yield recovery of DNA following DNA bisulfite conversion. Desulphonation and clean-up of the converted DNA is performed while bound to the MagPure Beads. The kit is optimized to minimize template degradation, loss of DNA during treatment and clean-up, and to provide complete conversion of unmethylated cytosines. Recovered DNA is ideal for PCR amplification for downstream analyses including endonuclease digestion, sequencing, microarrays, etc.

Features

- ❖ Complete, high-throughput, bisulfite conversion of DNA in less than 2 hours.
- ❖ A coupled heat denaturation/conversion reaction step streamlines the conversion of non-methylated cytosines into uracil.
- ❖ High throughput (96-well), automated desulphonation and recovery of bisulfite-treated DNA.
- ❖ Recovered DNA is ideal for downstream analyses such as PCR, endonuclease digestion, sequencing, microarrays, etc.

Reagent Preparation

- ### Preparation of CT Conversion Reagent

  The **CT Conversion Reagent** supplied within this kit is a solid mixture and must be prepared prior to first use. Prepare as follows:

1. Add 9 mL water, 3 mL of **Dilution Buffer**, and 500 µL **Dissolving Buffer** to a tube of **CT Conversion Reagent**.
2. Mix at room temperature with frequent vortexing or shaking for 15 minutes.

   Note: It is normal to see trace amounts of undissolved reagent in the **CT Conversion Reagent**. Each bottle of **CT Conversion Reagent** is designed for 96 separate DNA treatments.

   Storage: The **CT Conversion Reagent** is light sensitive, so minimize its exposure to light. For best results, the **CT Conversion Reagent** should be used immediately following preparation. If not used immediately, the **CT Conversion Reagent** solution can be stored overnight at room temperature, one week at 4°C, or up to one month at -20°C. Stored **CT Conversion Reagent** solution must be warmed to 37°C, then vortexed prior to use.

- **Preparation of Wash Buffer**

  Add 64 mL of 100% ethanol to the 16 mL **Wash Buffer** concentrate (D201-1) or 256 mL of 100% ethanol to the 64 mL **Wash Buffer** concentrate (D201-2) before use.

General Protocol

1. Add 130 µL of the **CT Conversion Reagent** to 20 µL of your DNA sample in a 96-well PCR plate. If the volume of the DNA sample is less than 20 µL, make up the difference with water. Mix the sample by pipetting the sample up and down.
2. Seal the plate, and place the plate in a thermal cycler and perform the following steps:

   Step1. 95 ^{o}C for 5 min
   Step2. 54 ^{o}C for 30 min
   Step3. 95 ^{o}C for 1 min
   Step4. 54 ^{o}C for 30 min
   Step5. 95 ^{o}C for 1 min
   Step6. 54 ^{o}C for 30 min
   Step7. 4 ^{o}C storage for up to 20 hours
   Note: The 4°C storage step is *optional*.
3. Add 600 µL of **Binding Buffer** and 20 µL of **MagPure Beads** to each well of a deep-well plate.
4. Transfer the samples from the 96-well PCR plate into the deep-well plate containing the **Binding Buffer** and **MagPure Beads**. Mix by pipetting up and down 3-6 times.
5. Let plate stand at room temperature for 5 minutes, then transfer plate to a magnetic stand for an additional 5 minutes or until beads pellet and supernatant is cleared. With the plate on the magnetic stand remove the supernatant and discard.
6. Remove the plate from the magnetic stand. Add 400 µL of **Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing the plate at 1,500 rpm for 30 seconds. Replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant.
7. Add 200 µL of **Desulphonation Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing for 30 seconds. Let plate stand at room temperature (20°C-30°C) for 15-20 minutes. After the incubation, replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant.
8. Add 200 µL of **Wash Buffer** to the beads. Re-suspend the beads by pipetting up and down or vortexing for 30 seconds. Replace the plate on the magnetic stand for 3 minutes or until beads pellet. Remove and discard supernatant. Repeat this wash step.
9. Dry the beads by placing the plate at 50°C for 10-30 min until the beads become brittle and brown.

10. Add 25 µL of **Elution Buffer** directly to the dried beads and pipette or vortex for 30 seconds to re-suspend.
11. Heat the elution at 50°C for 4 minutes then transfer the plate to the magnetic stand for 1 minute or until beads pellet. Pipette off the supernatant and transfer to a clean plate or tubes.