diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Rat GAPDH Primer Pair		
Cat. No: C17031046-50 C17031046-500	**Format:** 50 µl 500 µl	**Concentration:** 10 µM 10 µM

10 sets of our primer pairs: 50 µl (see our list)
500 µl

Description: The primer pair cat # pp-1046-050, -500 is specific to a promoter region of the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene from rat. The primers are optimized to be used in quantitative polymerase chain reaction (qPCR) (**Figures 1, 2**).

Application: The region amplified with GAPDH primer pair corresponds to a region of active chromatin. The primers can be used as a negative control to amplify DNA isolated by Methylated DNA immunoprecipitation (MeDIP) or Methylated DNA Capture (meDNA capture) (**Figure 3**). In Chromatin Immunoprecipitation (ChIP) assay, the primers can be used as a control representing active chromatin.

Expected PCR product size: 66 base pairs (bp)

Amplified locus: chr4:161285768-161285833

Specificity: rat positive

Format: In solution in MilliQ water at the concentration of 10 µM (each oligonucleotide primer is at the final concentration of 5 µM).

Storage: For long storage, store at -20°C. Avoid multiple freeze-thaw cycles.

Precautions: This product is for research use only. Not for use in diagnostic or therapeutic procedures.

Availability date: ..., 2009

Lot #: 001/ day of synthesis: May 13, 2009/ day of QC: May 18, 2009 /aliquoting: ..., 2009

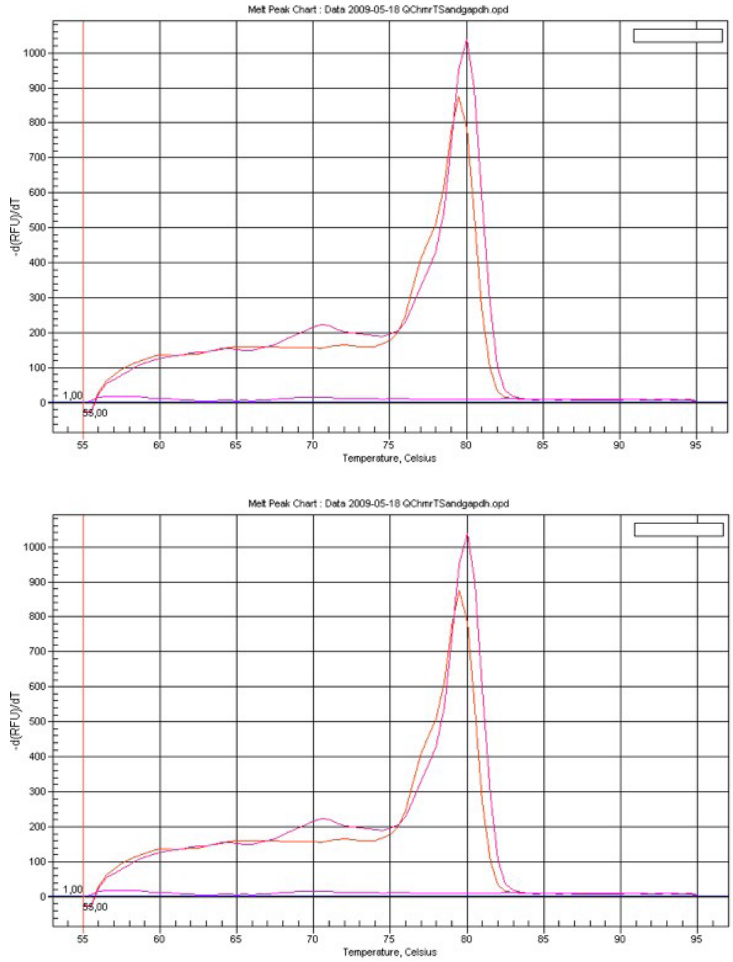

Figure 1

Melting curve of PCR product amplified with rat GAPDH primer pair (cat # pp-1046-050, -500).
Real-time qPCR was run in 25 µl of final volume with 1 µl of provided primers. PCR conditions were as follows: 95°C for 3 min, 40 cycles of [95°C for 15 seconds, 60°C for 45 seconds] and 1 cycle at 72°C for 2 min. The melting of the PCR product was performed from 55°C to 95°C, rising in 0.5°C increments.

Figure 2.

The PCR product amplified with rat GAPDH primer pair (cat # pp-1046-050, -500) as described in Figure 1 was analysed by electrophoresis (2% agarose gel stained with SYBR Safe). The left lane shows the 100 bp molecular weight ladder. The lane 1 shows the amplified region (66 bp). No amplification is found in negative control (no template DNA sample) (lane 2).

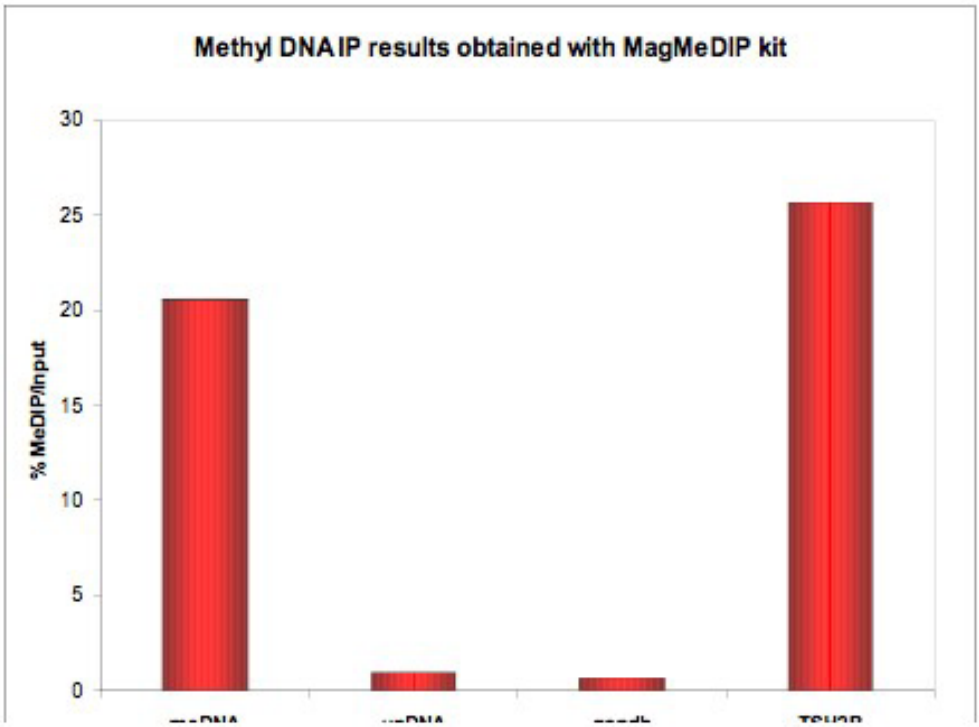

Figure 3

The region amplified with rat GAPDH primers (cat # pp-1046-050) corresponds to unmethylated locus.

Real-time PCR analysis was performed on DNA immunoprecipitated with MagMeDIP kit from Diagenode (cat # mc-magme-A10). Methyl DNA IP assay was performed using DNA from rat liver. The IP was performed including the kit's internal controls. The internal positive and negative controls included in the IP assay are methylated DNA (meDNA) and unmethylated DNA (unDNA). Immunoprecipitated DNA was amplified with PCR primers as indicated.

The expected results are as follows:

- Internal DNA controls
  "pos": meDNA control (positive signal is obtained for methylation)
  "neg": unDNA control (no signal is obtained for 0% methylation)

- Rat DNA
  "GAPDH promoter" (primer pair cat # pp-1046-050): no signal is expected as this region is not methylated.
  "TSH2B" (primer pair cat # pp-1043-050): a positive signal is expected as it is methylated region in somatic cells.