diagenode
A Hologic Company

TECHNICAL DATASHEET

PRODUCT NAME Rat TSH2B Primer pair			
Official full name: Histone H2B type 1-A **Other names:** Hist1h2ba, Th2b **Primary source:** RGD:3855			
Cat. No: pp-1043-050	**Size:** 50 µl	**Concentration:** 10 µM	**Lot #:** 001
Cat. No: pp-1043-500	**Size:** 500 µl	**Concentration:** 10 µM	**Lot #:** 001

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

Description: The primer pair cat # pp-1043-050, -500 is specific to a CpG region of the TSH2B gene from rat. The primers are optimized to be used in quantitative polymerase chain reaction (qPCR) (**Figures 1, 2**).

Application: The region amplified with TSH2B primer pair corresponds to genomic locus which is highly methylated in all somatic cells. The primers can be used as a positive 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 repressive chromatin.

Expected PCR product size: 99 base pairs (bp).

Amplified locus: chr17:48290238-48290336

Specificity: Rat: positive

Format: In solution in MiliQ 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.

References: [1] Choi YC, Chae CB, J Biol Chem. 1991 Oct 25; 266(30):20504-11
[2] Choi YC et al DNA Cell Biol, 1996, 15(6): 495-504

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 TSH2B primer pair (cat # pp-1043-050, -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

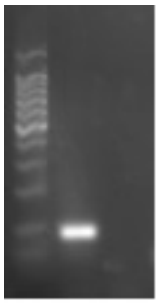

Figure 2
The PCR product amplified with rat TSH2B primer pair (cat# pp-1043-050, 500) as described in Figure 1 was analysed by electrophoresis (2% agarose gel stained with SYBR Safe). The left lane shows the 100 bp molecular weight ladder. The lane 1 shows the amplified region (99 bp). No amplification is found in negative control (no template DNA sample) (lane 2).

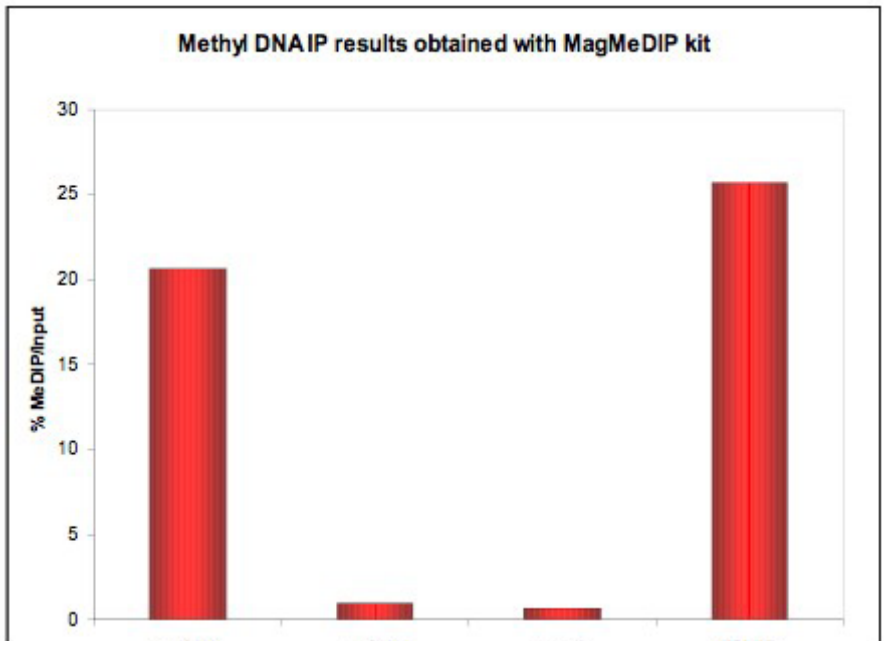

Figure 3

The region amplified with TSH2B primers (cat # pp-1043-050) corresponds to methylated 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.