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TECHNICAL DATASHEET

Human TSH2B promoter primer pair

Other names: STBP; H2BFU; bA317E16.3; HIST1H2BA

Primary source: HGNC:18730

Cat. No. C17011041

Size: 50 µl - 500 µl

Description: The primer pair Cat. No. C17011041 is specific to a CpG region of the TSH2B gene from human. 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 for repressive chromatin.

Amplicon length: 170 base pairs (bp).

Amplified region: chr6:25835060-25835229

Specificity: Human: positive

Format: 10 µM solution in MiliQ water (5 µM of each primer)

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

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Results

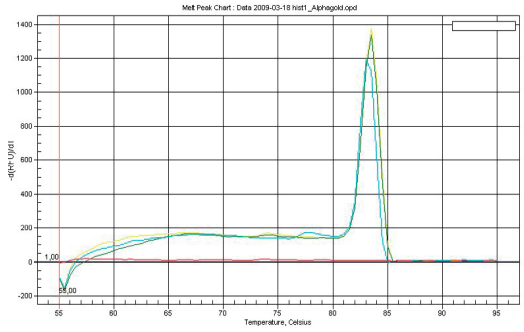

Figure 1

Melting curve of PCR product amplified with human TSH2B primer pair (Cat. No. C17011041). 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 curve analysis of the PCR product was performed by increasing the temperature from 55°C to 95°C in 0.5°C increments.

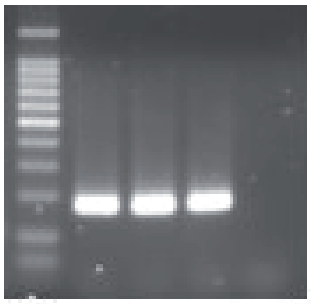

Figure 2

The PCR product amplified with human TSH2B primer pair (Cat. No. C17011041) 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 lanes 1, 2, 3 show the amplified region (170 bp). No amplification is found in negative control (no template DNA sample (lane 4)).

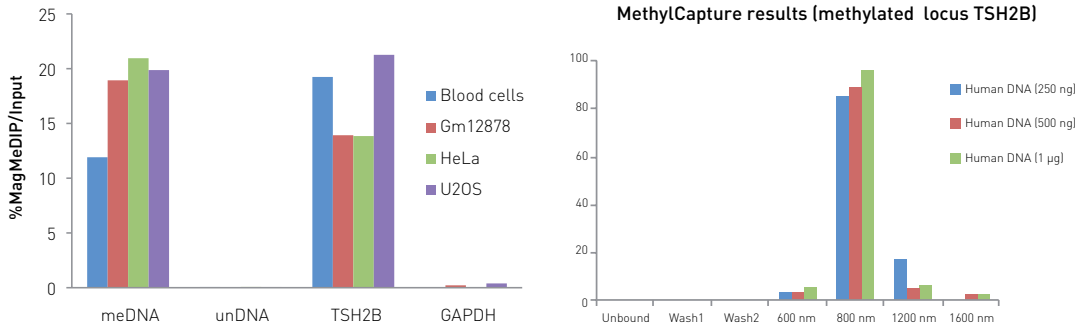

Figure 3. The region amplified with TSH2B primers (Cat. No. C17011041) corresponds to methylated locus.

Figure 3A: Methyl DNA IP assays were performed using DNA from blood, Gm12878, Hela and U20S cells and the MagMeDIP kit (Diagenode). The DNA was prepared with the GenDNA module. The IP was performed including the kit internal controls: together with the human DNA sample. The internal positive and negative DNA controls included in the IP assay are methylated DNA (meDNA) and unmethylated DNA (unDNA). The DNA is then isolated/purified using DIB. Afterwords, qPCR is performed using the primer pairs included in the kit.

Each "primer pair" targets a specific DNA and expected results are as follows:
Internal DNA controls:
- "meDNA": meDNA Positive control (positive signal is obtained for methylation).
- "unDNA": unDNA Negative control (no signal is obtained for 0% methylation).

Human DNA sample:
- "GAPDH promoter": no signal is expected as this region is not methylated.
- TSH2B: testis-specific H2B histone gene. A positive signal is expected as it is a methylated region. TSH2B gene is transcribed exclusively in testis. CpG sites of this gene are methylated in all somatic tissue but not in testis.

Figure 3B: Real-time PCR analysis with TSH2B primers was performed on DNA isolated by meDNA capture kit from Diagenode. Methylated DNA is bound to the complex MBD-beads and can be eluted by high salt solutions (600 mM -1.600mM). Unmethylated DNA does not bind to the complex MBD-beads. DNA from all fractions was amplified with TSH2B primers. The figure shows the significant enrichment for TSH2B locus in eluted fraction corresponding to methylated DNA but not in unbound and washed material.