

PRODUCT NAME			
Mouse Nkx2-5 promoter primer Pairs			
Official full name: NK2 transcription factor related, locus 5 (Drosophila)			
Other name: Csx, Nkx2.5, Nkx-2.5, tinman, Csx/Nkx2-5			
Primary source: MGI: 97350			
Cat. No: <b>pp-1033-050</b>	Size: 50 µl	Concentration: 10 µM	Lot #: 001
Cat. No: <b>pp-1033-500</b>	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-1033 (-050, -500) is specific to a DNA region in the mouse Nkx2-5 gene promoter [1]. These primers can be used to amplify DNA isolated by chromatin immunoprecipitation (ChIP). Primers are optimized to be used in quantitative polymerase chain reaction (qPCR) (**Figures 1, 2 and 3**). See overview below.

**Expected PCR product size:** 138 base pairs (bp).

**Specificity:** Mouse: positive  
Other species: not tested

**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.

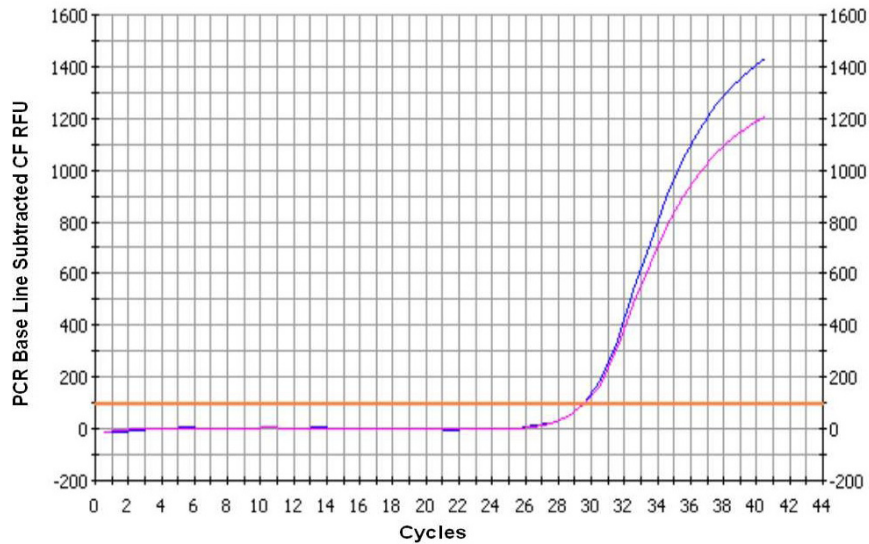
**Precautions:** This product is for research use only. Not for use in diagnostic or therapeutic procedures.

**References:** [1] O'Neill L.P., VerMilyea M.D. and Turner B.M. (2006) Nat. Genet. 38(7):835-41.  
[2] Akazawa H. and Komuro I. (2005) Pharmacol. Ther. 107(2): 252-68.

**Availability date:** September 03, 2007

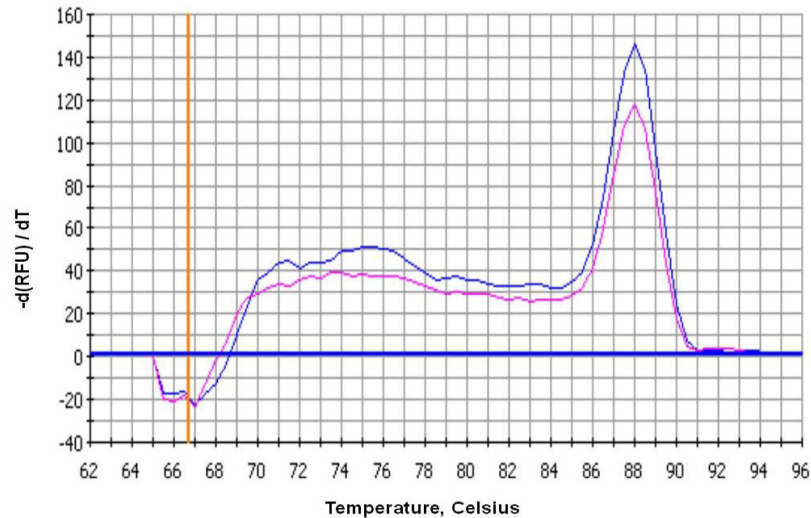
**Last data sheet update:** September 14, 2007

**Lot #:** 001/ day of the synthesis: May 25, 2007/ day of QC: August 20, 2007/ aliquoting day: August 24, 2007



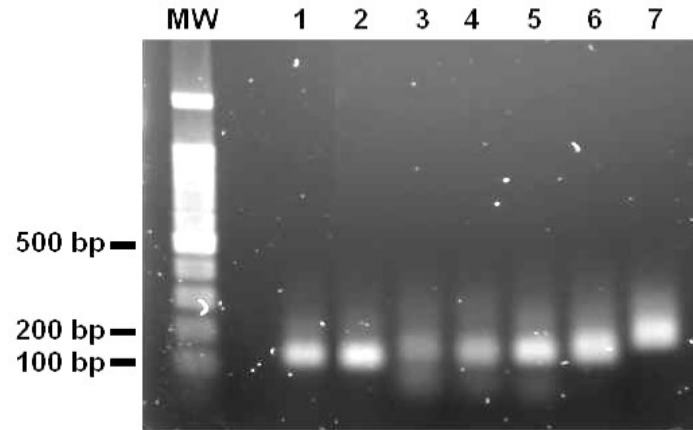
**Figure 1**

DNA from mouse fibroblast 3T3 cells was analyzed in duplicate by real-time PCR starting from 5  $\mu$ l of DNA template (0.03  $\mu$ g/ml) using the Diagenode primers to amplify a region in the mouse Nkx2-5 gene promoter (cat#: pp-1033-050, -500). One  $\mu$ l of provided primer pairs is used by PCR of 25  $\mu$ l final volume. A Real-Time PCR Detection System and iQ SYBR Green have been used. qPCR conditions used are as follows: 95°C for 3 minutes, 41 cycles of: [95°C for 60 seconds, 60°C for 60 seconds and 72°C for 90 seconds]. Duplicates are shown in blue and pink Threshold position is in orange.



**Figure 2.**

Melting curves obtained with the primers cat#: pp1033 [-050, -500] used in the above qPCR. Conditions were 60 cycles of 65°C for 1 minute and increment of 0.5°C per cycle. Duplicates are shown in blue and pink.



**Figure 3**

qPCR products were analysed by electrophoresis (1.5% agarose gel) stained with SYBR Safe and illuminated with UV light. The left lane shows molecular weight markers (MW) that decrease in size by 100 bp. Different qPCR products using different primer pairs which are available at Diagenode were tested: 1: primers for mouse Cdx2 gene promoter (pp-1025-050, -500), 2: primers for mouse Hhex gene promoter (pp-1027-050, -500), 3: primers for a coding region of the mouse Hhex gene (pp-1028-050, -500), 4: primers for a 3' region of the mouse Hhex gene (pp-1029-050, -500), 5: primers for mouse Nkx2-5 gene promoter (pp-1033-050, -500), 6: primers for mouse Cfc1 gene promoter (pp-1035-050, -500), 7: primers for a coding region of the mouse Cfc1 gene (pp-1036-050, -500). For more details about other primer pairs, see data sheet.

**Overview:** Csx/Nkx2-5 is a member of the NK homeobox gene family that is conserved in evolution and acts as a DNA-binding transcriptional activator. Csx/Nkx2-5 was identified as a potential mammalian homologue of NK4 (tinman) gene of the *Drosophila*. "Csx" is the acronym of cardiac-specific homeobox and represents its distinctive biological significance in myocardial cell lineage. "Nkx2-5" has originated from a taxonomical standpoint that it is the fifth vertebrate gene identified in the NK-2 homeobox gene family. Currently, both names are widely adopted, and a double name "Csx/Nkx2-5" is used in this paper. As expected as a homologue of tinman, Csx/Nkx2-5 is highly expressed in the early heart progenitor cells in both primary and secondary heart fields during murine embryogenesis and continues to be expressed at a high level in the heart through adulthood. Especially, a transient elevation of Csx/Nkx2-5 expression is observed in specialized myocardial conduction cells during the period of conduction system formation, suggesting a significant role of Csx/Nkx2-5 in the development of the conduction system [2].