

## Product Information

### Control Primer Set for *Arabidopsis* UBC Gene

Catalog Number **C3240**  
 Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

### Product Description

This product consists of a primer set specific for a portion of the *Arabidopsis* ubiquitin-conjugating enzyme gene (UBC, At5g25760). It is intended for use as an internal control in RT-PCR analysis of *Arabidopsis* gene expression.

The UBC primer set is designed as a control for transcripts that typically demonstrate medium to low abundance. Two additional control primer sets are also offered by Sigma. The nuclear cap-binding protein CBP20 primer set, Catalog Number C3115, is designed as a control for medium to low abundance transcripts, and the Actin-2 primer set, Catalog Number C3615, for high abundance transcripts.

### Components

Each tube contains enough primer (forward or reverse) for 100 RT-PCR reactions (50  $\mu\text{l}$  reaction volume)

- Forward Primer –  $T_m = 62\text{ }^{\circ}\text{C}$  based on % GC method in 1 M salt concentration; 20  $\mu\text{M}$  in TE buffer
- Reverse Primer –  $T_m = 64\text{ }^{\circ}\text{C}$  based on % GC method in 1 M salt concentration; 20  $\mu\text{M}$ , in TE buffer

### Precautions and Disclaimer

This product is for R&D use only. Not for drug, household or other uses. Consult the MSDS for information regarding hazards and safe handling techniques.

### Storage/Stability

Store at  $-20\text{ }^{\circ}\text{C}$ . Stable at  $-20\text{ }^{\circ}\text{C}$  for at least 1 year; can be frozen and thawed at least 10 times.

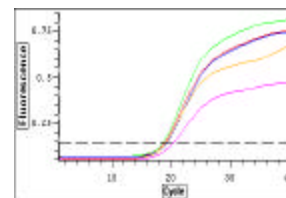
### Recommended PCR Procedure

| Step                   | Temperature           | Time       |
|------------------------|-----------------------|------------|
| For Cycles 1-30:       |                       |            |
| Denaturation           | 94 $^{\circ}\text{C}$ | 15 sec     |
| Annealing              | 65 $^{\circ}\text{C}$ | 45 sec     |
| Extension              | 72 $^{\circ}\text{C}$ | 1 min      |
| Plate Read (real-time) | 80 $^{\circ}\text{C}$ | 1 sec      |
| Incubate               | 72 $^{\circ}\text{C}$ | 10 min     |
|                        | 4 $^{\circ}\text{C}$  | Indefinite |

### Product Profile

The primer set has been validated by quantitative RT-PCR with total RNA isolated from five different *Arabidopsis* tissues, leaf, root, stem, flower, and silique. The size of the amplicon produced by this set of primers is 219 bp. The forward primer sequence is 5'-TCAAATGGACCGCTCTTATC-3' and that of the reverse primer is 5'-CACAGACTGAAGCGTCCAAG-3'.

The following figure shows expected performance of the primer set on different *Arabidopsis* tissues. Total RNA samples were treated with amplification grade DNase I, Catalog Number AMPD1, to remove genomic DNA. Equal amounts of total RNA, based on OD measurements at 260 nm, were added in each RT-PCR reaction. Standard RT-PCR was conducted using the JumpStart™ RED HT RT-PCR Kit, Catalog Number J3520, whereas real-time RT-PCR data was generated using the SYBR® Green Quantitative RT-PCR Kit, Catalog Number QR0100.



## References

1. Tomasz Czechowski, T., et al. Genome-Wide Identification and Testing of Superior Reference Genes for Transcript Normalization in Arabidopsis. *Plant Physiol.* **139**, 5-17 (2005).
2. Lovatt, A., et al. Validation of quantitative PCR assays. *BioPharm*, March 2002, p. 22-32.
3. Bustin, S. A., Quantification of mRNA using real-time reverse transcription PCR (RT-PCR): trends and problems. *J. Mol. Endocrinol.*, **29**, 23-29 (2002).
4. Ginzinger, D. G., Gene quantification using real-time quantitative PCR: an emerging technology hits the mainstream. *Exp. Hematol.*, **30**, 503-512 (2002).

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DC,PHC 12/05-1

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