

## MGB Eclipse® PCR Reagent Kit

Catalog Number **M4568**  
Storage Temperature  $-20\text{ }^{\circ}\text{C}$

## TECHNICAL BULLETIN

The MGB Eclipse PCR Reagent Kit is optimized for quantitative PCR mediated Gene Expression Analysis in conjunction with MGB Eclipse Probe Systems. It is pre-formulated with necessary buffers and deoxynucleotides and when combined with JumpStart™ Taq DNA Polymerase creates a ready-to-use 2× PCR master mix. The kit also contains dUTP for the optional addition of uracil-N-glycosylase (UNG) to prevent carry-over contamination from previous target amplification. The MGB Eclipse PCR Reagent Kit can be used for all real-time PCR applications with the exception of MGB Eclipse Probe Systems designed for allelic discrimination or single nucleotide polymorphism analysis. For such applications, MGB Eclipse PCR Reagent Kit for SNPs, Catalog Number M4693, is recommended.

Once the 2× master mix is prepared according to the procedure below, combine and run with probes, primers, and template according to the protocol provided with your MGB Eclipse Probe System from Epoch Biosciences.

The MGB Eclipse PCR Reagent Kit is:

- The ideal reagent for high performance gene expression analysis and general sequence detection using MGB Eclipse Probe Systems.
- Scalable for different reaction volumes and flexible for optional use of UNG.
- Prevents non-specific product formation when combined with the hot start mechanism of the JumpStart Taq antibody and allows assembled PCR reactions to be placed at room temperature for up to 2 hours without compromising the performance.
- Can save a significant amount of preparation time, reduce the risk of contamination from multiple pipetting steps, and provide consistent batch-to-batch and reaction-to-reaction performance when performing large numbers of PCR reactions.

### Components/Reagents

Sufficient reagents are provided for either 200 reactions or 400 reactions, using a 50  $\mu\text{L}$  reaction volume.

- JumpStart Taq DNA Polymerase, Catalog Number D5568, 2.5 units/ $\mu\text{L}$
- MGB Eclipse PreMix for Gene Expression, Catalog Number M4318  
Combine with the recommended volume of JumpStart Taq DNA Polymerase, Catalog Number D5568; to prepare a 2× PCR ReadyMix™ with 10 mM  $\text{MgCl}_2$ , 0.5 mM dUTP, 0.25 mM each of dATP, dCTP, and dGTP, 20 mM Tris-HCl, pH 8.3, and 100 mM KCl.

### Materials and Reagents Required but not Provided

- Water, PCR Reagent, Catalog Number W1754
- MGB Eclipse Probes & Primers from Epoch Biosciences
- DNA template
- Dedicated pipettes
- PCR pipette tips
- PurePak PCR microtubes, thin-walls  
0.2 ml, Catalog Number P 3114  
0.5 ml, Catalog Number P 3364  
or plates for specific thermal cyclers
- Thermal cycler
- Heat labile uracil N-glycosylase (UNG), also uracil DNA glycosylase, available from several suppliers.

### Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

### Storage/Stability

The kit can be stored at  $-20\text{ }^{\circ}\text{C}$  for up to a year. There is no detectable loss of performance after 10 freeze-thaw cycles.

### Procedure

Optimal concentrations of template DNA, MgCl<sub>2</sub>, KCl, and PCR adjuncts, as well as pH, are often target specific. Optimization may be required for specific template and primers. The following procedure serves as a reference.

1. Add the following reagents to a 0.2 ml or 0.5 ml thin-walled PCR microtube or plate well.

| Volume | Reagent                                  | Final Concentration   |
|--------|--|---|
| 24 µL  | MGB Eclipse Premix for Gene Expression   | 10 mM Tris-HCl, pH 8.3, 50mM KCl, 5 mM MgCl <sub>2</sub> , Nucleotides: 0.25 mM dUTP and 0.125 mM dATP, dCTP and dGTP |
| 1 µL   | JumpStart Taq DNA Polymerase 2.5 unit/µL | 0.05 units/µL   |
| 2.5 µL | Epoch Primer                             |   |
| 2.5 µL | Epoch Probe                              |   |
| 2 µL   | Template DNA                             | ~0.2 ng/µL  |
| 1 µL   | UNG                                      | (optional)  |
| 17µL   | Water                                    | (if UNG used; add additional 1 µl if UNG not used)  |
| 50 µL  | Total Volume                             |   |

**Note 1:** It is recommended that the user make a master mix of JumpStart Taq DNA Polymerase and MGB Eclipse PreMix for Gene Expression, as well as a template-primer master mix, for each dilution of template when performing multiple PCR reactions.

**Note 2:** For the best performance in gene expression studies when the target sequence exceeds 60% GC, we recommend the use of a PCR enhancer, e.g., DMSO, betaine or MasterAmp™ 10x PCR Enhancer. The PCR enhancer should be added as recommended by the manufacturer.

2. Mix by gently vortexing and briefly centrifuge to collect all components at the bottom of the tube
3. Optimal cycling parameters vary with PCR composition and thermal cycler. It may be necessary to optimize the cycling parameters to achieve maximal product yield and/or quality.

### Typical cycling parameters

|                        |                |             |
|------------------------|----------------|-------------|
| UNG reaction*          | 37-50 °C       | 1 - 10 min. |
| Initial denaturation   | 95 °C          | 2 min.      |
| <b>45 cycles:</b>      |                |             |
| Denaturation           | 95 °C          | 5 sec.      |
| Annealing              | 56 °C          | 20 sec.     |
| Extension              | 76 °C          | 20 sec.     |
| <b>Data collection</b> | Annealing step |             |
| Hold                   | 4 °C           |             |

\* Follow the supplier's recommended conditions.

### References

1. Dieffenbach, C., and Dveksler, G., (Eds),. PCR Primer: A Laboratory Manual, 2<sup>nd</sup> ed., Cold Spring Harbor Laboratory Press, 2003.
2. Rees, W. A., et al., *Biochemistry*, **32**, 137-144 (1993).
3. Don, R. H., et al., *Nucleic Acids Res.*, **19**, 4008 (1991).
4. Kwok, S., and Higuchi, R., *Nature*, **339**, 237-238 (1989).

## Troubleshooting Guide

| Problem   | Possible Cause   | Solution  |
|---|--|---|
| No fluorescent signal is observed. No PCR product is detected by agarose electrophoresis.             | A PCR component is missing or degraded   | A positive control should always be run to insure components are functioning. A checklist is also recommended when assembling reactions.  |
|   | Thermal parameters are not optimal   | An initial denaturation of greater than two minutes will not activate enzyme further, and is likely to degrade the template, primers, enzyme and/or dNTPs, thereby reducing PCR efficiency.   |
|   | Magnesium concentration is sub-optimal   | Titrate trial PCR adding MgCl <sub>2</sub> concentrations in 0.2 mM increments  |
|   | Detection step is set incorrectly.   | Make sure that detection is toggled on, and that the cycle step selected is appropriate (Data acquisition occurs at the annealing step with Epoch products)   |
|   | There is not enough template.  | Repeat the reaction with a higher concentration of template.  |
|   | The template is of poor quality.   | Evaluate the template integrity by agarose gel electrophoresis. It may be necessary to repurify template using methods that minimize shearing and nicking.  |
|   | PCR inhibitors are present in the sample.                                      | Dilute the sample two-fold and ten-fold in PCR Reagent water, and repeat.   |
|   | Incorrect primers were used.   | Check the primer sequences.   |
| No fluorescent signal is observed, but a specific PCR product is detected by agarose electrophoresis. | Detection step is set incorrectly.   | Insure the detector or channel settings are consistent with probe label. Make sure that detection is toggled on, and that the cycle step selected is appropriate. (Data acquisition occurs at the annealing step with Epoch products) |
| C <sub>t</sub> values are not related to the DNA template concentration.                              | Magnesium concentration is sub-optimal.  | Titrate trial PCR adding MgCl <sub>2</sub> concentrations in 0.2 mM increments  |
|   | Primer-dimers were amplified   | Reduce the primer concentration by 50-95%. This may improve specificity.  |
|   | Incorrect primers were used.   | Check the primer sequences.   |
|   | The template concentration is too high.  | Reduce the concentration of the template in the PCR reaction. This is especially important when applying baseline correction.   |
|   | The template concentration is too low.   | Low or no template often increases primer-dimer (non-specific product) formation.   |
| Gel electrophoresis shows multiple bands, or a primer dimer band (~50 bp)                             | Quantitative PCR instrument is contaminated.                                   | Decontaminate the instrument according to the manufacturer's instructions.  |
|   | There was crossover contamination of specific and/or nonspecific PCR products. | Take special precautions to avoid crossover contamination of PCR reactions, including primer-dimer artifacts. <sup>4</sup> Consider using UNG to remove crossover contamination.  |

**Related Products**Reagents

- Betaine solution, 5 M, Catalog Number B0300
- Dimethyl sulfoxide, PCR Reagent, Catalog Number D9170

Equipment

- PCR Workstation, 120 V, Catalog Number Z376213
- PCR Workstation, 240 V, Catalog Number Z376221

Books

- PCR: Essential Data Series, C. Newton, Ed., John Wiley and Sons, Ltd., 1995  
Catalog Number Z364916
- PCR: A Practical Approach, M. J. McPherson, et al., Eds., IRL Press at Oxford University Press, 1991. Catalog Number P7186
- PCR 3: PCR In Situ Hybridization, C.S. Herrington and J. J. O'Leary, Eds., IRL Press at Oxford University Press, 1997. Catalog Number Z378399
- PCR Cloning Protocols: 2<sup>nd</sup> ed., B. Y. Chan and H. W. Janes, Eds., Humana Press, 2002.  
Catalog Number P8117
- PCR In Bioanalysis: Methods in Molecular Biology, Vol. 92, S. J. Smeltzer, Ed., Humana Press, 1998.  
Catalog Number Z379603
- PCR Primer: A Laboratory Manual, 2<sup>nd</sup> ed., C. Dieffenbach and G. Dvekster, Eds., Cold Spring Harbor Laboratory Press, 2003.  
Catalog Number Z701270
- PCR Protocols: A Guide to Methods and Applications, M. A. Innis, et al., Eds., Academic Press, 1990. Catalog Number P8177
- PCR Protocols for Emerging Infectious Diseases, D. H. Persing, Ed., American Society for Microbiology, 1996. Catalog Number Z369918
- PCR Sequencing Protocols, R. Rapley, Humana Press, 1996. Catalog Number Z373818
- PCR Strategies, M. Innis, et al., Eds., Academic Press, 1995. Catalog Number Z364452
- PCR Technology, Current Innovations, 2<sup>nd</sup> ed., T. Weissensteiner, et al., Eds., CRC Press, 2003.  
Catalog Number Z704318

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