LAMDA BIOTECH

PRODUCT INFORMATION

Catalog No.: D502R

Product Name: **EvaGreen Direct-qPCR Kit**

Description: **EvaGreen Direct-qPCR Kit** contains all the reagents needed for a quick preparation of genomic DNA and the qPCR master mix for **SYBR green real-time PCR assay**. Any type of tissues can be used for this kit, such as mouse tails and nail tips, and other animal tissues and cells; plant leaf, root or seeds; bacteria, fungi and other samples. The fluorescent dye can be used in the same way as SYBR green but with a higher sensitivity and heat stability.

Kit Contents:

Size:	100 rxns
DNA Extraction Solution **A**	13 ml
DNA Extraction Solution **B**	1.5 ml
2X qPCR Universal Green MasterMix	1.0 ml

Storage: The whole kit can be stored at 4°C for up to three months or at -20°C for long-term.

General Protocol

I. DNA Sample Preparation:

1. Place the sample into a PCR tube:
   - For mouse tissues from tail, ear or nail: 1-3 mm in length or diameter.
   - Animal tissues: 1-3mg.
   - Cultured cells: 10µl of cell culture.
   - Plant materials: 1-3mg (approximately the size of a sesame seed)
   - Other samples: similar amount or volume as above.
2. Add 100µl of the **DNA Extraction Solution A** into the tube containing the sample.
3. Heat the sample for 10 minutes at 95°C. This can be easily done in a PCR machine.
4. Take out the sample and add 10µl of the **DNA Extraction Solution B**.
5. Mix well with vortex or by shaking the tube a few times
6. The sample DNA is now ready for real-time PCR or stored at or below 4°C for future applications.

Note:

A. The sample can be centrifuged briefly before use.

B. Use only the supernatant for qPCR and avoid any undigested tissue or debris.

II. Real-time PCR:

Prepare a reaction mixture using the following:

Components	Volume 20µl	Final Concentration
2X qPCR Universal Green MasterMix	10.0µl	1x
Primer A	Variable	100-500nM
Primer B	Variable	100-500nM
Sample DNA	1.0µl	*<500ng*
RNase-free Water	Up to 20µl	-
Total Volume	20µl	-

Perform real-time PCR according to your favorable program, or try the following program.

Step	Temperature	Duration – Standard	Duration - Fast	Cycles
Enzyme Activation	95°C	10min	10min	Hold
Denature	95°C	15sec	3sec	40
Anneal/extend	60°C	60sec	30sec	
Melting Curve	According to the instrument guidelines			

Recommendations for Optimal Results

- Aliquot reagents to avoid contamination and to avoid repeated freeze-thaw cycles
- EvaGreen qPCR Master Mix components are light sensitive; avoid exposure to light
- If needed, ROX reference can be used by adding the ROX dye to the 2X qPCR Universal Green MasterMix.
- Start PCR as soon as the reaction mixture is prepared and always keep the reaction mixture chilled in an ice box prior to PCR reactions.

Troubleshooting: Problems and Solutions

Q1. Samples are not completely digested or dissolved?

A1. Samples are not expected to be digested or dissolved completely. Do not worry. Sufficient DNA will be released for PCR without complete digestion of the samples.

Q2. There is little or no real-time PCR signal is detected?

A2. Please consider one of the following:

a) Make sure that there are no PCR components missed.
b) More PCR cycles may be needed.
c) Primers may not be designed optimally.
d) Adjust the real-time PCR parameters to find out the optimal condition for your primers.
e) Too much sample may have been used. In that case, the samples can be easily diluted 10 times with H_2O or 10mM Tris-HCl buffer, pH 8.5.

Q3. A high background/noise signal?

A3. Adjust your annealing temperature or other parameters for your PCR program.

Q4. Negative control shows false positive signal?

A4. Reagents or your samples may be contaminated.

END