AFLP® Core Reagent Kit

Cat. No.: 10482-016 **Size: 50 templates**
Store at -20°C.

Description:
Amplified Restriction Fragment Polymorphism (AFLP®) is a powerful technique used for DNA fingerprinting (1,2). The AFLP® Analysis Systems contain both the AFLP® Core Reagent Kit and an AFLP® Primer Kit designed for a specific application. The AFLP® Core Reagent Kit contains reagents for template preparation, including restriction enzymes, adapters, ligase and appropriate buffers. Primers, a manual and other reagents required for the AFLP® amplification reactions are contained in the AFLP® Primer Kits.

Components	Part No.	Amount
EcoR I/*Mse* I [1.25 units/µl each in 10 mM Tris-HCl (pH 7.4), 50 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.1 mg/ml BSA, 50% glycerol (v/v), 0.1% Triton® X-100]	51114	100 µl
5X Reaction Buffer [50 mM Tris-HCl (pH 7.5), 50 mM Mg-acetate, 250 mM K-acetate]	51115	250 µl
Distilled Water	50837	1.25 ml
Adapter/Ligation Solution [*EcoR* I/*Mse* I adapters, 0.4 mM ATP, 10 mM Tris-HCl (pH 7.5), 10 mM Mg-acetate, 50 mM K-acetate]	51116	1.2 ml
T4 DNA Ligase [1 unit/µl in 10 mM Tris-HCl (pH 7.5), 1 mM DTT, 50 mM KCl, 50% (v/v) glycerol]	Y01301	50 µl
TE Buffer [10 mM Tris-HCl (pH 8.0), 0.1 mM EDTA]	50282	4.5 ml
Tomato DNA (100 ng/µl)	51117	10 µl
Arabidopsis DNA (100 ng/µl)	51118	10 µl

Quality Control:
All components were tested in the following protocol using the control DNA. Template prepared in this protocol was amplified using the primers and reagents provided in AFLP® Primer Kits and displayed the specified DNA fingerprint.

Instructions for Use:(For more detailed information, please refer to the AFLP® Analysis System manual)

1. Add 100-500 ng of your sample DNA (volume of sample DNA must be 18 µl or less) to a 1.5-ml microcentrifuge tube. Add 5 µl 5X Reaction Buffer, 2 µl *EcoR* I/*Mse* I and Distilled Water to a final volume of 25 µl.
2. For control reaction: Combine 2.5 µl control DNA, 5 µl 5X Reaction Buffer, 2 µl *EcoR* I/*Mse* I and 15.5 µl Distilled Water to a 1.5-ml microcentrifuge tube.
3. Mix gently and collect reaction by brief centrifugation. Incubate the mixture 2 h at 37°C.
4. Incubate the mixture for 15 min at 70°C to inactivate the restriction endonucleases. Place the tube on ice and collect contents by brief centrifugation.

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5. To a tube of digested DNA from Step 3, add 24 µl Adapter/Ligation Solution and 1 µl of T4 DNA Ligase.

6. Mix gently at room temperature, centrifuge briefly to collect contents, and incubate at 20°C for 2 h.

7. Perform a 1:10 dilution of the ligation mixture as follows:
   Take 10 µl of the reaction mixture and transfer to a 1.5-ml microcentrifuge tube.

   Add 90 µl TE buffer and mix well.

8. Diluted ligation mixture is then used for pre-amplification using AFLP® primers and other reagents provided in AFLP® Primer Kits.

9. The unused portion of the reaction mixture may be stored at -20°C.

References:

1. Zabeau, M. and Vos. P. (1993) *European Patent Application*, publication number EP 0534858.
2. Lin, J.J. and Kuo, J. (1995) *Focus® 17*, 66.