MDA phi29 Kit

Description:

MDA phi 29 Kit contains phi29 DNA Polymerase, that catalyzes a highly processive DNA synthesis (up to 70,000 base insertions per binding event) coupled to strand displacement activity[1]. It possesses an inherent 3'-5' exonuclease activity[2] acting preferentially on single-stranded DNA. The kit is designed to perform MDA (multiple displacement amplification) reactions. The Modified Random Hexamer Primers included in the kit are protected against degradation by the 3'-5' exonuclease activity of the phi 29 DNA Polymerase.

Components:

Component	Cat. No. E1402-01 25 reactions	Cat. No. E1402-02 125 reactions
phi29 MDA DNA Polymerase	25 µl	125 µl
10x phi29 MDA Reaction Buffer	1 x 100 µl	1 x 500 µl
10mM dNTPs Mix	50 µl	250 µl
Modified Random Hexamer Primers 500µM	50 µl	250 µl
20mg/ml BSA nonacetylated	10 µl	50 µl
1M DTT	25 µl	125 µl

References:

1. Blanco, L. et al. (1989) J. Biol. Chem., 264, 8935-8940.
2. Garmendia, C. et al. (1992) J. Biol. Chem., 267, 2594-2599.

EURx Ltd. 80-297 Gdańsk Poland ul. Przyrodników 3, NIP 957-07-05-191, KRS 0000202039
www.eurx.com.pl, orders@eurx.com.pl, *tel.* +48 58 524 06 97, *fax* +48 58 341 74 23

Protocol for MDA reaction (20µl vol.):

1. Perform DNA sample denaturation:

a) Combine 2 µL of Modified Random Hexamer Primers with up to 12 µL of puriefied DNA sample, mix well, settle by centrifuging,

b) Denature at 95°C for 3 minutes, then put on ice immediately to cool.

2. Combine the components of the reaction on ice. Add enzyme as the last component of the reaction.

Mix the following components to set up a 20 µl reaction:

Reaction components:	**Final volume: 20 µl**
DNA denatured with Modified Random Hexamer Primers	up to 14 µl
10x phi29 MDA Reaction Buffer	2 µl
20 mg/ml BSA nonacetylated	0,2 µl
10 mM dNTPs Mix	2 µl
phi29 MDA DNA Polymerase	1µl
Sterile H_2O	up to 20 µl

3. Incubate the mixure at 30°C for 1,5-12 hours. (Note 1)

4. Heat inactivate by placing the reaction at 65°C for 10 minutes.

Notes:

1. The incubation time of the reaction is dependent on the initial amount of template DNA. Extending the reaction time may be beneficial when the amount of starting DNA is very small.
2. For best polymerase activity, it is recommended to supplement the reaction with freshly diluted DTT to a final concentration of 4 mM.

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