Direct WGA Kit

Cat#: orb533392 (User Manual)

Applications:

• Geno type analysis

• PCR and real-timePCR

• Construction of genomic library

Description: Direct WGA Kit is a complete system for whole genome amplification from various tissues or samples directly without DNA purification processes. Very little amount of samples, several miligram or microliter volume, are required for the direct WGA. About 10 µg DNA products could be obtained in a standard reaction. The enzyme mix and buffer system are designed to tolerate against most amplification inhibitors found in crude samples. Phi29 DNA polymerase, the major polymerization enzyme of this kit, isothermally amplifies the genomic DNAs included in the samples with multiple displacement mechanism. Phi29 DNA polymerase could produce DNA strand up to 70 kb long with high fidelity. All required components including enzymes, buffers, dNTPs, randomprimers, and sample pretreatment reagents are supplied in this kit. The amplified DNA products could be applied for successive PCR, geno typing and library construction.

• Fast and uniform amplification across entire genome

• MultipleDisplacementAmplificationbyPhi29DNApolymerase • Direct WGA from Whole blood, animal tissues, plant leaves and seeds,clinical & forensic sample[Saliva,Buccalswab,Hairroot, Bloodstain(toilet paper or paper)]

Content:

Component	PCR-382S	PCR-382L
1 M DTT	100 µl	500 µl
PBS Buffer	20 µl	100 µl
DB	1.0 ml	5 x 1.0 ml
NB	40 µl	200 µl
Primer Mix	20 µl	100 µl
Enzyme Mix	20 µl	100 µl
Reaction Buffer	240 µl	1.2 ml
dNTP Mix (each 10 mM)	40 µl	200 µl

Preparation Procedure

1. Preparation of DM Buffer

• for one reaction mix 50 µl DB with 5 µl 1 M DTT (for blood samplesmix5µlDBwith0.5µl1MDTT) • Pleasenote: DM Buffer should be fresh lyprepared for use

2. Sample Preparation

For Blood Samples

• Add 1µl of PBS Buffer to 0.5-1 µl of whole blood sample.

• Add 1.5µl of DM Buffer and mix by pipetting.

• Incubate on ice for 10min.

• Add 1.5µl of NB.Briefly vortex and spin down.

For Animal tissue

• Transfer 50µl of DM Buffer in to a1.5ml microtube.

• Addatissueslicesizeofabout5mmintotheDMbuffer. Briefly mix by vortexing and spin down.

• Incubate at room temperature for 10min.

• Transfer2µl of the supernatant into a new1.5ml microtube.

• Add 2µl of NB. Mix by pipetting and spin down.

For Plant Leaves or Seeds

• Transfer 50µl of DM Buffer in to a1.5ml microtube.

• Add a plant leaf cut size of about 5mm or several small(<1mm size) pieces of cracked plant seeds into the DM buffer. Briefly mix by vortexing and spin down.

• Incubate at room temperature for 10min.

• Transfer2µlofthesupernatantintoanew1.5mlmicrotube.

• Add2µl of NB.Mix by pipetting and spin down.

3. Preparation of the mix

component	20 µl assay
Reaction Buffer	12 µl
dNTP Mix (10 µM)	2 µl
Primer Mix	1 µl
Enzyme Mix	1 µl
PCR-grade water	fill up to 20 µl

4. Incubation

• Incubate at 30°C for 1.5hours and inactivate theenzyme at 65 °C for 3min.

• Please note: Perform the reaction at at hermalcycler or incubator. Water-bath is not recommendable. For PCR, use 1-2 µl of10-fold diluted product with distilled water. If the PCR is not successful,it is recommended touse 1-2 µl of undiluted product as PCR template.

5. Storage

• Store amplified DNA at-20°C.