# 96-Well Plasmid Kit Protocol

# -Combined centrifuge and vacuum method

## **Description:**

This protocol is for high copy plasmid, not exceeding 1.5ml of culture. Low copy plasmid could be lysed in 1.5ml tube first, and then transfer the supernatant onto the DNA binding plate on Step 6.

- Add provided RNase A to the MX1 Buffer and store at 4°C
- If precipitates have formed in the MX2 Buffer, warm up in a 37°C incubator or water bath until the precipitates dissolve
- Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume)
- Additional requirements: centrifugation system for 96well plates, 2 ml collection plates, absolute ethanol

#### Protocol:

#### Step1

#### Harvesting

- Transfer 1.5 ml of cultured bacterial cells to a 2 ml collection plate.
- Centrifuge for 3 minutes at 3,000 x g.
- · Remove the medium.

#### Step 2

#### Resuspension

- Add 100  $\mu l$  of MX1 Buffer (RNase A added) to each well of the plate and
- Resuspend the cell pellet completely by vortex 2-5 min.

#### Step 3 Lysis

- Add 100  $\mu l$  of MX2 Buffer to each well and mix gently by shaking.
- Let stand at room temperature for 2 minutes or until the lysate clears.

#### Step 4

#### Neutralization

- Add 150 µl of MX3 Buffer and mix immediately by shaking gently.
- Centrifuge for 10 minutes at 3,000 x g. Following centrifugation, a denatured protein pellet and clear lysate (supernatant) will be present in each well.

### Step 5

#### Lysate clear by filtration (optional)

- If clear cell lysate can not be effectively formed from step 4 then do the following:
- Place a 2 ml collection plate on the base of the vacuum manifold and place a filter Plate on top of the vacuum manifold.
- Carefully transfer ONLY supernatant from Step 4 to the filter Plate (approximately 200-300 μl).
- Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.

# Step 6

# DNA Binding

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- Place a 2 ml collection plate on the base of the vacuum manifold and place a Plasmid Binding Plate on top of the vacuum manifold.
- Carefully transfer filtrate from Step 5 to the Plasmid Binding Plate (approximately 200-300  $\mu$ l).

Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.

#### Step 7 Wash

- Turn off the vacuum pump and add 800 μl of Wash Buffer to each well of the Plasmid Plate.
- Apply vacuum at 10 inches Hg for 5 minutes or until the wells have emptied.
- Turn off the vacuum pump and add 800  $\mu$ l of Wash Buffer (ethanol added) to each well of the Plasmid Plate to wash again.
- Apply vacuum at 10 inches Hg for 3 minutes or until the wells have emptied.
- To dry the plate:
  - Centrifuge for 10 minutes at 3,000 x g and then dry the plate at 37C for 15 minutes to remove any ethanol residue.
  - o Or use a heated speedvac at 60C for 8 minutes
  - o Do not over dry the plate!

#### Step 8

#### DNA Elution

- Transfer the Plasmid Plate to a new 96 well collection plate.
- Add 80-120  $\mu l$  of Elution Buffer or TE into the center of each membrane matrix.
- Let stand for 2-5 minutes or until the Elution Buffer or TE is absorbed.
- Centrifuge for 10 minutes at 3,000 x g to elute the purified DNA. You should expect 60-80ul of DNA.
- Load 3 ul plasmid on agarose gel for concentration and quality.

# Appendix: components ordering information

# Filter plate: Cat. No. 2021F-002 Size: 2 plates

DNA Binding Plate:
Cat. No. 2020-001 Size: 1 plate

#### Proteinase K:

Cat. No. B108-1000 Size: 1 g

Rnase A:

Cat. No. B408-1G Size: 1 g