KIAGENE FANAVAR

# KIASMID PLUS
# Plasmid DNA Isolation Kit (solution based)

**Cat. No:**

**Eco Kit FPKT020.0025**
**Mini Kit FPKT020.0050**
**Midi Kit FPKT020.0100**
**Maxi Kit FPKT020.0200**

## Contents:

| Components | 25 preps | 50 preps | 100 preps | 200 preps |
|---|---|---|---|---|
| PSB1 Buffer (blue) | 2.5 ml | 5 ml | 10 ml | 20 ml |
| PSB2 Buffer (orange) | 5 ml | 10 ml | 20 ml | 40 ml |
| PSB3 Buffer (yellow) | 3.75 ml | 7.5 ml | 15 ml | 30 ml |
| PSB4 Buffer (green) | 12.5 ml | 25 ml | 50 ml | 100 ml |
| PEB | 1 ml | 2 ml | 4 ml | 8 ml |
| Rnase A 10mg/ml | 100ul | 200ul | 400ul | 800ul |

Kit storage:
⚠ This kit should be stored at room temperature.
⚠ RNase A should be stored at -20 °C
If properly stored, all kit components are stable until the expiration date printed on the label.
⚠ After adding RNaseA to PSB1 buffer Store the PSB1 at +2 to +8°C

## Additional Equipment and Reagent required

•Absolute ethanol
•Ethanol 70%
• Standard tabletop microcentrifuge capable of 13,000 x g centrifugal force
• Microcentrifuge tubes, 1.5 ml, sterile

## Application:

Isolation of up to 15µg purified plasmid DNA from bacterial cultures, which may be used directly in downstream applications such as restriction enzyme digestion, PCR, cloning, sequencing, in vitro transcription, or labeling reactions. The procedure is optimized to achieve reliable results within 90 min.

## Handling Requirements and Safety Information

⚠ *All solutions are clear and should not be used when precipitates have formed. Warm the solutions at +15 to +25°C or in a 37°C water bath until the precipitates have dissolved.*

⚠ *PSB4 contain Phenol which is an irritant and toxic.*

⚠ *Do not allow PSB4 Buffers to touch your skin, eyes, or mucous membranes. If contact does occur, wash the affected area immediately with large amounts of water.*

⚠ *If you spill the reagent, dilute the spill with water before wiping it up.*

⚠ *Do not use any modified ethanol.*

⚠ *Do not pool reagents from different lot numbers.*

⚠ *Immediately after usage, close all bottles in order to avoid leakage, varying buffer concentrations or buffer conditions.*

⚠ *After first opening store all bottles in an upright position.*

⚠ *Do not allow the PSB4 Buffers to mix with sodium hypochlorite found in commercial bleach solutions. This mixture can produce a highly toxic gas.*

⚠ Wear protective disposable gloves, laboratory coats and eye protection, when handling samples and kit reagents.

⚠ Do not contaminate the reagents with bacteria, virus, or nucleases. Use disposable pipets and nuclease free pipet tips only, to remove aliquots from reagent bottles.

## preparation procedure:

Working Solution Preparation

⚠ **add RNase A to PSB1 Buffer completely Dissolve the RNase A by vortexing. store the PSB1 buffer at 4 °C.**

## Protocol

**1. Sample preparation**
Pellet the bacterial cells from **1-5 ml of E. coli culture** (OD600= 1.5-5 per ml) by centrifuge in **8000g for 1 min**. Discard the supernatant (Do not use more highly concentrated sample)

**2. Add 100 µl chilled PSB1 Buffer (with RNase A)** to the bacterial pellet. Resuspend the bacterial pellet by **vortex for 30 s.**

**3. Add 200 µl PSB2 Buffer** to the mixture. Mix gently by **inverting the tube 5 to 10 times (Do not vortex). Incubated at room temperature for 1 min** (Do not incubate more).

**4. Add 150 µl chilled PSB3 Buffer**. Mix gently by **inverting the tube 5 to 10 times (Do not vortex)**. **Incubated on ice for 5 min** (The solution should become cloudy and a flocculent precipitate should form).

**5.** Centrifuge at **Maximum speed for 5 min at +4°C** and transfer entire supernatant into a new 1.5 ml microtube.

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**6. Add 500 µl PSB4** and Centrifuge at **Maximum speed for 2 min at +4°C**

**7.** Transfer upper solution layer into a new microtube. **Add 2-Fold Absolute ethanol and incobate 2 min at RT.**

**8.** Centrifuge at **Maximum speed for 5 min at +4°C.** (a thin and white pellet should be seen in bottom of microtube) Discard supernatant carefully.

**9. Add 1ml Ethanol 70%** and invert several. Centrifuge at **Maximum speed for 2 min at +4°C.**

**10.** Discard supernatant carefully. to remove residual ethanol, **incubate microtube at +55°C on hot plate for 5-10 min.**

**11. Add 50 µl PEB Buffer or sterile DDW** on pellet and pipetage gently.

**12.** Check **5-10 µl of extracted plasmid DNA** by 1% agarose gel electrophoresis. Store Plasmid DNA at -20˚C