

Name: **InsertChecker - Clone Screening Solution**

Cat. #: **H101**

Size: 2 x 1 ml / vial, 100 preps (20ul / prep)

Ship & Store at RT

**Description:** InsertChecker® simplifies colony screening by combining bacterial lysis, plasmid extraction, and gel loading into one step—cutting workflow time from 24 hours to just 0.5 hours.

**Application:** InsertChecker® releases sufficient plasmid DNA directly from individual E. coli colonies while also functioning as a DNA gel loading buffer. The lysate can be loaded directly onto an agarose gel for electrophoresis. Insert-containing colonies can be easily identified by comparing their plasmid bands with the parental vector. This product is for Research Use Only.

**Key Features:**

- Rapid insert screening — no culture or plasmid prep.
- Process many colonies **at once.**
- Save Time - Cut Workflow from **24 Hours to 0.5 Hour.**
- Non-toxic: no phenol or chloroform.
- Stable at room temperature.

**Protocol:**

Overview: InsertChecker® combines bacterial lysis and DNA gel loading buffer functions in one step. It has been optimized to efficiently release plasmids from E. coli, and the resulting lysate can be directly loaded onto a DNA gel without further processing.

1. Sample Preparation

Add 20 µl of InsertChecker® to each PCR tube, microplate well, or other microtube. (Example: For screening 9 colonies, prepare 10 tubes — 9 for colonies and 1 for vector control.)

2. Colony Collection and Lysis

Using a 20 µl pipette tip, gently touch a bacterial colony (~1 mm diameter) and transfer it into the tube containing 20 µl of InsertChecker®. Mix by pipetting up and down to lyse the cells. If the colony is larger, use 25 µl of InsertChecker®.

### 3. Gel Electrophoresis

After lysis, load 20 µl of the lysate directly onto a 1% agarose gel for electrophoresis.

The lysate contains plasmid DNA, E. coli genomic DNA, and rRNA (16S, 23S, 5S), so an external DNA marker is not required.

Use the original circular vector (without insert or enzyme digestion) as the empty vector control.

Colonies containing inserts can be identified by size differences on the gel. Mark positive colonies on the plate.

### 4. Notice:

A: Because E. coli genomic DNA may adhere to pipette tips and pull the sample out of the well, slide the tip along the side of the gel well while withdrawing it. This helps embed the genomic DNA into the well and ensures complete sample loading.

B: This product is preferably used for sub-cloning of pUC plasmid systems. For overnight cultured bacteria, take 100ul of culture medium, centrifuge for 3 minutes, and take the precipitate for screening.

### Three Easy Steps:

1. Pick a colony and mix with 20 µl InsertChecker solution.
2. Load 20 µl of the lysate onto a 1% agarose gel.
3. Run electrophoresis to identify insert-positive clones by band size.

