ALiCE®
Protein Expression Made Easy

Short protocol for protein expression with ALiCE®

Important notices

RNase contamination leads to lower or no protein yields. Only use **RNase-free filter tips** and **wear gloves at all times!**

ALiCE® requires oxygen during the whole reaction time for a successful reaction. **Do not seal reaction vessels!**

1) Choose reaction vessel

ALiCE® Tubes **or** 96 half well plate with lid.

2) Prepare DNA

Thaw DNA template at room temperature, mix briefly. Do not heat-treat.

Positive control plasmids pALiCE01 and pALiCE02 are supplied ready-to-use at 250 ng/µL. See instruction manual for additional information on template preparation.

3) Thaw ALiCE® Reaction mix

Thaw ALiCE® reaction mix in a waterbath at room temperature (20 - 25 °C). **Do not vortex ALiCE® Reaction mix!** Start reactions within 30 min after thawing.

Freeze remaining lysate at -80°C. **Do not use liquid nitrogen.** Avoid more than one freeze-thaw cycle.

4) Reaction assembly and reaction

4a) ALiCE® Tubes

Assemble at room temperature:

Component	Volume
ALiCE® Reaction mix	48 µL
pALiCE vector / DNA template	2 µL
Total volume	**50 µL**

Only use the supplied punctured caps to close ALiCE® Tubes.

4b) 96 half well plates*

Assemble at room temperature:

Component	Volume
ALiCE® Reaction mix	48 µL
pALiCE vector / DNA template	2 µL
Total volume	**50 µL**

Important: Add ~ 75 µL of water in the interwell space.

Note: When using pALiCE plasmids with different inserts, dilute or concentrate to the same final molarity of 5 nM. DNA concentration may significantly affect protein yield.

$$\text{mass}_{\text{your DNA template}} = \frac{\text{length}_{\text{your complete vector}}\,[\text{bp}]}{3000\,[\text{bp}]} \times 0{,}5\ \mu\text{g}$$

* For best results, use plates from Greiner Bio-One, Art. No. 675086

5) Reaction parameters

4a) ALiCE® Tubes

Incubate the ALiCE® Tubes in an orbital table-top shaker at 700 rpm and 25 °C for 48 h. We recommend a 3 mm shaking diameter for optimal results.

4b) 96 well plates

Incubate the plates in an orbital shaker at 500 rpm and 25 °C for 48 h. We recommend a 12.5 mm shaking diameter and a controlled humidity of > 70 % for optimal results.

Due to the long incubation time, evaporation may occur. Please refill the reaction vessel with RNase-free water to 50 µL after the reaction is completed.

Do not seal reaction vessels or plates!

When using pALiCE01 as a positive control, a yellow color should be visible in the Reaction Mix after 48 hours.

Usage notes

- Template DNA is a common source of RNase contamination. Purification of DNA with a procedure based on anion exchange chromatography is therefore highly recommended. Alternatively, the template DNA can be purified by phenol-chloroform extraction prior to use with ALiCE®. Also, RNase inhibitor can be added before the reaction.
- When using other reaction conditions or other volumes than stated above or hindering the oxygen transfer by sealing the plates, protein yields will be diminished.
- For additional information on plasmid preparation, protein purification (SDS PAGE) and microsome targeting, please refer to the instruction manual on our website: www.leniobio.com

For *in vitro* / research use only!

The kit is shipped frozen on dry ice, please check if the contents are still frozen upon delivery. Contact us immediately if any issues with delivery have occurred.

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