PROTEIN EXTRACTION FROM E.COLI USING PRECELLYS® EVOLUTION

Institut Pasteur, PFPR, Paris, France

CONTEXT

The lab is responsible for screening a large number of E.coli culture conditions for the optimization of soluble protein production. Due to the flexibility and efficiency of the Precellys® Evolution, 3 volumes (1mL, 5mL, 10mL) of high concentrated E.coli cells were effectively lysed to extract soluble proteins for analytical or purification purposes. Different quantities of glass beads in Precellys® lysing tubes were evaluated.

MATERIALS

- Precellys® Evolution
- Precellys® lysing kit: VK01_2mL (KT03961-1-005.2); Empty tube_7mL (KT03961-1-404.7) + 3.5g glass beads 0.1mm (KT03961-1-104.BK); Empty tube_7mL (KT03961-1-404.7) + 2.4g glass beads 0.1mm; Empty tube_15mL (KT03961-1-406.15) + 7g glass beads 0.1mm; Empty tube_15mL (KT03961-1-406.15) + 4.8g glass beads 0.1mm (KT03961-1-104.BK)
- Sample: E.coli cells (DMSO1230) were normalized to an OD600 of 50, and subsequently loaded into a Precellys® lysis kit (1mL into 2mL tubes; 5mL into 7mL tubes; 10mL into 15mL tubes)

CONTACT
Email: sample-prep@bertin.group
www.bertin-technologies.com

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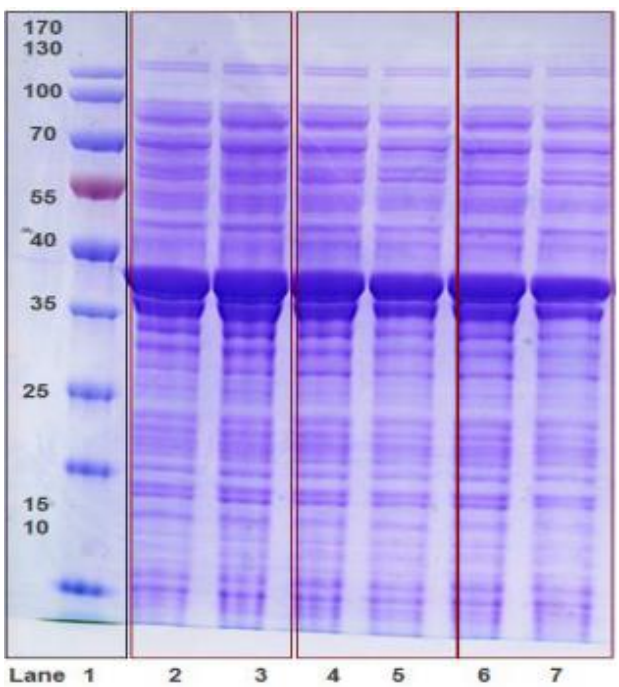
PROTOCOL

Precellys® Evolution (stored in a cold room): 9000rpm; 6x30sec (60s break) for 2mL and 7mL tubes; 9900rpm; 6x30sec (60s break) for 15mL tubes.

Analysis: after homogenization, the tubes were centrifuged for 15min at 5000 rpm. The supernatant contains the soluble protein fraction. The protein extracts were separated by SDS-PAGE, followed by Coomassie blue staining.

RESULTS

The gel picture obtained after homogenization on the Precellys® Evolution (Figure 1) shows an efficient extraction of protein into multivolume Precellys® lysing tubes (2mL, 7mL and 15mL). A higher quantity of glass beads improves the extraction of soluble protein.



The gel was stained with Coomassie Blue. Lane 1: molecular weight standard; Lanes 2 and 3: 1mL E.coli prep/0.7g glass beads_2mL; Lane 4: 5mL E.coli prep/3.5g glass beads_7mL tube); Lane 5: 5mL E.coli prep/2.4g glass beads_7mL tube; Lane 6: 10mL E.coli prep/7.0g glass beads_15mL tube; Lane 7: 10mL E.coli prep/4.8g glass beads_15mL tube.

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CONCLUSION

The homogenizer Precellys® Evolution is suitable and convenient for high concentration (OD600=50) of E.coli cells lysis. Due to the flexibility of the Precellys® Evolution (2.0, 7.0 and 15mL Precellys® tubes), a scale-up volume of soluble proteins extracts can be carried out efficiently.

The Cryolys® cooling unit can be used to prevent degradation of thermo-sensitive samples.

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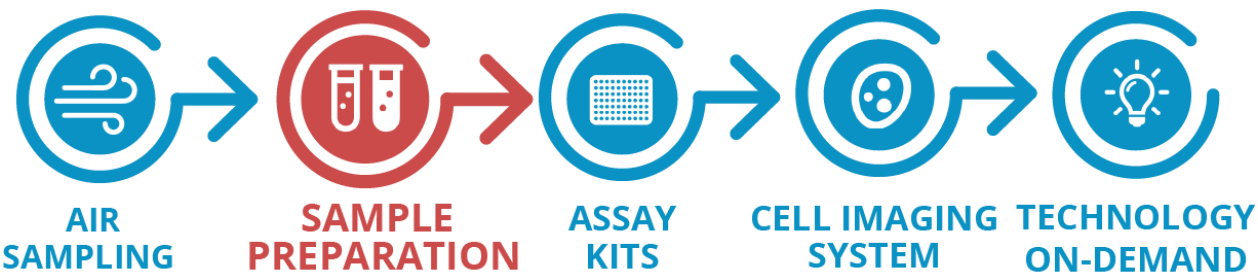

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