Performing Protein Expression, Purification, and Quantification on a Robotic Liquid Handling Platform



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INTRODUCTION

There is a growing interest in automating the workflow for protein expression, purification, and quantification. A typical application is to more efficiently generate a pool of therapeutic monoclonal antibodies (mAb) with modified variable regions in the early stages of drug discovery and development. To address this interest, we tested the capabilities of Opentrons robotic liquid handlers OT-2 and Flex to perform several critical tasks in the workflow of antibody production.

RESULTS

HeLa cells transfected with the vector carrying IgG cDNA by automated Protein Expression protocol on OT-2 platform, and success of transfection confirmed by GFP expression visualized by fluorescence microscopy (Figure 1)







Opentrons FlexTM



OT-2

Figure 1. HeLa cells were seeded in a 6-well plate at 70% confluency and then cultured overnight. Transfection master mix per well was prepared by mixing DNA (2 µg) with FuGene Transfection Reagent (6 µL) in serum free (DMEM 100 µL). After 10-minute incubation at room temperature, Protein Expression protocol was run on OT-2 equipped with HEPA module. Transfected cells were cultured for 72 hours, and images captured by EVOS M7000 (Thermo Scientific, Waltham, MA) and culture medium harvested.

Supernatants harvested from transfected HeLa cell culture processed to collect secreted IgG by automated Protein Purification protocol on Flex platform, and purified IgG confirmed by SDS-PAGE and Western Blot (Figure 2).



METHOD

PROTOCOL PROTEIN EXPRESSION

Platform: Opentrons OT-2 with HEPA Module

Workflow:

- Day 1: Prepare HeLa cells in a 6-well plate (Induction at 37°C and 5% CO₂ off deck)
- Day 2: Prepare DNA/transfection reagent mixture (pRABBIT IgG IRES-EmGFP Positive Control Vector from Thermo Scientific, Waltham, MA, and FuGENE HD Transfection Reagent from Promega, Madison, WI) and add above mixture to HeLa cell culture (Induction at 37°C and 5% CO₂ off deck)
- Day 5: Harvest culture medium (subjected to protein purification)

PROTOCOL PROTEIN PURIFICATION

Platform: Opentrons Flex with Heater Shaker Module and Magnetic Module

Workflow:

- 1. Prepare Dynabeads Protein G (Thermo Scientific, Waltham, MA) with equilibration buffer
- 2. Incubate and agitate samples/beads mixture to capture target protein (agitation for 2 hours)3. Wash x2

4. Elute target protein

Final products subjected to SDS-PAGE and Western Blot or protein quantification

PROTOCOL PROTEIN QUANTIFICATION

Figure 2. Media harvested 72 hours after transfection were processed on Flex for IgG isolation using Dynabeads Protein G, and eluates subjected to SDS-PAGE and Western Blot. Upper: purified vs. unpurified medium; Lower: the presence of rabbit IgG detected by using IRDye® 680RD Goat anti-Rabbit IgG (LI-COR Biosciences, Lincoln, NE)

BCA protein assay for purified IgG in the final product performed by automated Protein Quantification protocol on Flex platform (Figure 3).



Platform: Opentrons Flex with Temperature Module

Workflow:

Prepare working reagent (BCA Protein Assay from Thermo Scientific, Waltham, MA)
Prepare working plate by adding working reagent, samples and standards into a 96-well plate
Incubation (37°C, 30 minutes)

Readouts obtained by measuring absorbances at 560 nm on a plate reader off deck, and protein levels estimated by referencing the standard curve.

Protocol scripts available in open-source Opentrons Protocol Library (https://library.opentrons.com):

- Cell Culture Preparation (up to 4 plates)
- DNA Transfection (up to 4 plates)
- Dynabeads for Immunoprecipitation
- Dynabeads for Immunoprecipitation Reagents in 15 mL tubes
- BCA Protein Assay

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CONCLUSION

A panel of automated protocols utilizing robotic liquid handlers streamlines critical steps in the workflow for recombinant mAb production: protein expression, purification and quantification. Our preliminary work has demonstrated both the feasibility and advantages of automating the processes, which include minimal hands-on time and the capability of high throughout sample preparation.