Cell Signaling Assay
Hardware integration on the Beckman Coulter Biomek

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1. OVERVIEW

Cell Signaling is a complex mechanism that directs basic cellular processes and corresponding actions. Errors in cell signaling responses cause the tissue's environmental operating point to change and causing multiple disease states.

Traditional approaches to the study of cell signaling pathways involve laborious and rigorous sample preparations. The complex nature of these assays tends to limit the throughput of the studies, and results may depend on the technique and the manual skills of the investigator.

Automation of assay preparation is the intended resolution for this complex preparation burden.

2. INTRODUCTION

The current manual methodology for performing cell signaling assays is a complex process that requires the use of multiple daughter tubes, repetitive pipetting steps, temperature cycling and multiple washing steps. In an experimental study involving multiple patient samples, the scientist bears a significant managerial burden in order to maintain the integrity of the results. The automation solution uses a Beckman Coulter Biomek NXp laboratory workstation, fitted with standard Automated Labware Positioners (ALPS) and custom adapters. The resulting automated assay is a method implemented in the Biomek programming environment to distribute the reagents, aliquot the sample, incubate the reaction plate, wash the resulting preparation, and re-suspend the cell pellet. Because the incubation temperature affects the reaction rates (kinetics) for the various signaling epitopes, it is a principle component of the reaction variation. The hardware integration and optimization of two key areas of the assay is hereby discussed.

3. METHODS

•Materials used

- •Specimen type – Peripheral whole blood from healthy donors
- •Reagents
  - •Activators: Phorbol 12-myristate 13-acetate (PMA) or Lipopolysaccharide (LPS);
  - •10% Formaldehyde; Triton X-100; Methanol (MeOH)
  - •1X Phosphate Buffered Saline (PBS);
  - •Wash Buffer: 4% Fetal Bovine serum in 1x PBS;
  - •Signaling Antibodies

Manual Assay

Start: Aliquot 100 µL sample

Cell Activation:
- •Add activator reagent to reaction tube and mix
- •Incubate at 37º C for a desired time

Cell Fixation:
- •Add 65 µL Formaldehyde and mix
- •Incubate at room temperature for 10 minutes

Cell membrane permeabilization:
- •Add 1 mL Triton X-100 and mix
- •Incubate at 37º C for 15 minutes
- •Cell wash 1
  - oAdd 1 mL cold wash buffer and mix
  - oPellet cells by centrifugation
  - oRemove supernatant
- •Re-suspend by adding 1 mL of cold 50% methanol in PBS
- •Incubate at 0º C for 10 minutes

Cell Staining:
- •Cell wash 2: Add 2 mL cold wash buffer and mix. Pellet cells by centrifugation
- •Cell wash 3: Same as Cell wash 2
- •Add antibodies
- •Incubate at room temperature for 30 minutes
- •Cell wash 4: Same as Cell wash 2
- •Re-suspend by adding 350 µL of 0.5% Formaldehyde in PBS

Stop: Transport to Cytometer

Automated Assay

Start: Aliquot 100 µL sample

Cell Activation:
- •Add activator reagent to reaction tube and shake
- •Incubate at 37º C for a desired time

Cell Fixation:
- •Add 65 µL Formaldehyde and shake

Incubate at 37º C for 3 minutes

Cell membrane permeabilization:
- •Add 1 mLTriton X-100 and shake
- •Incubate at 37º C for 15 minutes
- •Cell wash 1
  - oAdd 1 mL cold wash buffer and shake
  - oPellet cells by centrifugation
  - oRemove supernatant
- •Re-suspend by adding 1 mL of cold 50% methanol in PBS
- Incubate at room temperature for 10 minutes

Cell Staining:
- •Cell wash 2: Add 2 mL cold wash buffer and mix. Pellet cells by centrifugation
- •Cell wash 3: Same as Cell wash 2
- •Add antibodies
- •Incubate at room temperature for 30 minutes
- •Cell wash 4: Same as Cell wash 2
- •Re-suspend by adding 350 µL of 0.5% Formaldehyde in PBS

Stop: Transport to Cytometer

4. HARDWARE INTEGRATION

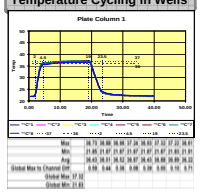
An onboard Peltier module is integrated onto the Biomek platform as a source of incubation temperature cycling and control. The Peltier module provides for a rapid temperature increase to 37• C without overshoot, a rapid temperature decrease to ambient without undershoot, and efficient thermal transfer to minimize thermal gradients at the reaction well plate. A multi channel thermocouple probe developed to allow efficient characterization of the Peltier module and reaction plate. This tool used to monitor and collect temperature data for each well in the reaction plate. The Peltier system was characterized by, transferring a test volume to each well, running a test temperature cycling protocol, and collecting the resulting temperature data. All forty eight wells in the reaction plate were characterized using this protocol. The data provided by the initial and each subsequent characterization allowed the Peltier system to be optimized. The optimization process involved, constructing, changing or adjusting physical system elements and modifying the temperature system control parameters

The fluid transfer system provides fast and accurate low and medium volume dispenses. The low volumes are 1uLand 2 µL, while the medium volumes are in the 1 mL range. The solution that addresses these transfers will preferably use existing hardware resources. The optimization of the low volume transfers will also satisfy the needs for the medium volumes. After evaluating various syringe configurations, the 1 mL syringe was selected as optimal for this implementation. The low volume reagent transfer needs are met by the use of low volume pipetting techniques and templates within the Biomek programming method.

Deck and ALPS

Labware

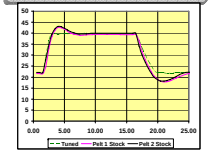
Temperature Cycling in Wells



Automated Assay Unit

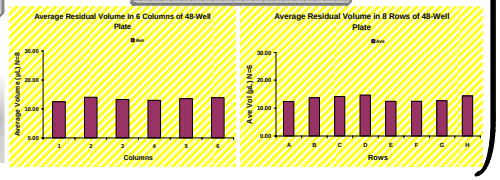
Temperature Cycling Peltier

Tuned vs. Stock Peltiers

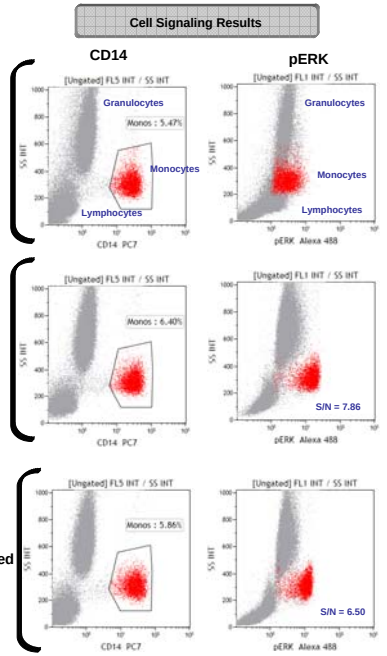


Gallios Flow Cytometer

Residual Volume Measurements



Cell Signaling Results



5. CONCLUSIONS

The final Peltier system module achieved a well behaved system with no significant undershoot or overshoot, rapid temperature increase and decrease rates and minimal plate thermal gradient. The final system configuration also achieved a low supernatant dead volume of less than 20 µL.

When compared to the manual method, the automated method generates similar results in a faster and reproducible fashion. This relieves the burdens on the researcher for the intensely manual labor of assay preparation resulting in increased assay throughput.