KingFisher instruments and Dynabeads magnetic beads

The key combination for successful cell isolation, seamlessly integrated with protein isolation and analysis

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Introduction

Blood is a very complex matrix consisting of numerous cell types with various functions. Understanding their functions is of great importance, but blood samples can be challenging to study. Reducing sample complexity by isolating cells of interest or removing unwanted cells is therefore a critical step prior to downstream examination.

Invitrogen™ Dynabeads™ magnetic beads are uniform, nonporous, monodisperse superparamagnetic polystyrene beads that are widely used in various applications. The beads provide consistent physical and chemical properties that have been instrumental in providing high-performance cell isolation products targeting cells of the immune system and other cell types, for the last 30 years.

Here we have integrated an automated cell isolation approach with an automated protein isolation method using Dynabeads magnetic beads. The Thermo Scientific™ KingFisher™ Flex, Duo Prime, and Apex instruments were used for automated cell and protein isolation. Protein separation, transfer, and labeling were performed using Invitrogen™ Bolt™ gels, the Invitrogen™ iBlot™ 2 Gel Transfer Device, and the Invitrogen™ iBind™ Western Device, respectively. This workflow reduced the total process time from 2–3 days to less than 7 hours (Figure 1).

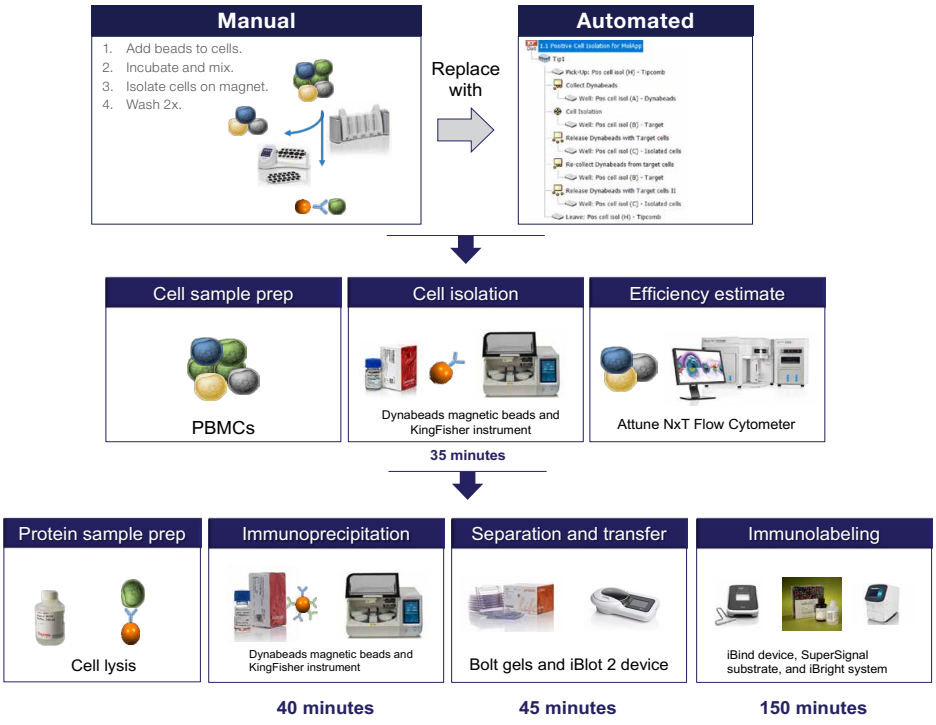

Figure 1. Workflow for automated cell and protein capture followed by rapid protein analysis.

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Materials and methods

The Jurkat cell line was used for optimizing the cell isolation script on the KingFisher instruments. Biologically relevant cells were isolated from peripheral blood mononuclear cells (PBMCs) for downstream protein analysis. Flow cytometry was used to estimate the isolation efficiency by measuring target cell depletion. Invitrogen™ Dynabeads™ CD3 (Cat. No. 11151D), Dynabeads™ CD4 (Cat. No. 11145D), Dynabeads™ CD8 (Cat. No. 11147D), Dynabeads™ CD14 (Cat. No. 11149D), and Dynabeads™ CD19 Pan B (Cat. No. 11143D) were used for cell isolation. KingFisher instruments with Thermo Scientific™ BindIt™ software (4.0) were used for automation. Manual isolation was performed following the protocol in the package insert.

Isolated T cells were lysed and processed for immunoprecipitation (IP) and western blotting using NP40-based cell lysis buffer. IP was performed using Invitrogen™ Dynabeads™ Protein G (Cat. No. 10003D) conjugated with Invitrogen™ CD81 Monoclonal Antibody (Cat. No. 10630D). A KingFisher instrument with BindIt software (4.0) was used for automation. Downstream analysis included standard electrophoresis using Bolt gels and western blotting transfer using the iBlot 2 Gel Transfer Device. The iBind Western Device was used for immunolabeling of CD81 protein. TrueBlot™ anti–mouse IgG, HRP conjugate (Rockland Immunochemicals), was used as a secondary antibody. Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate (Cat. No. 37071) was used as a substrate.

Results

Optimization of incubation time during cell isolation

Immunomagnetic cell separation with Dynabeads magnetic beads can be achieved with most types of starting samples, such as whole blood, washed blood, buffy coat, bone marrow, mononuclear cells (MNCs), or single-cell suspensions from various tissues. The incubation time for binding target cells to magnetic beads commonly varies between users. Here, CD3-positive Jurkat cells were chosen as the starting material to determine the incubation time required for efficient positive isolation. The use of a cell line eliminated potential blood donor variation. The Jurkat cells were incubated with Dynabeads magnetic beads for 10 min, 20 min, 30 min, and 60 min (Figure 2). The isolation efficiency was estimated by flow cytometric analysis by measuring the remaining cells in the depleted fraction. The results demonstrated that isolation efficiency using a KingFisher instrument with 20 min or 30 min incubation was comparable to manual isolation. Interestingly, the data showed that most of the binding takes place within the first 10 min, and increasing the incubation time from 30 min to 60 min did not increase yield (Figure 2).

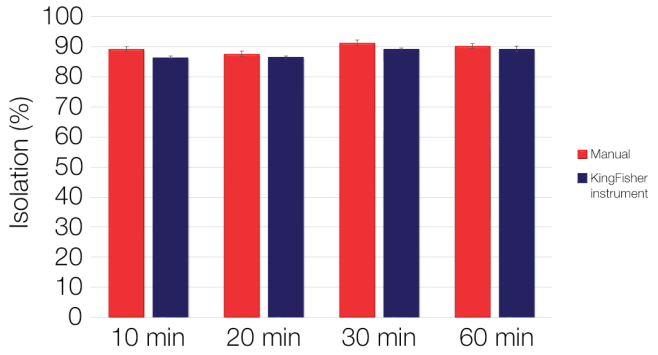

Figure 2. Incubation time for isolation of CD3-positive Jurkat cells. Jurkat cells were incubated with Dynabeads CD3 for 10 min, 20 min, 30 min, or 60 min using the KingFisher Duo Prime instrument. Cell isolation results were compared with those of the manual approach. Flow cytometry was used to estimate the isolation efficiency.

Optimization of mixing during cell isolation

To avoid loss of target cells and help ensure the highest yield, it is critical to select optimal mixing conditions during the cell isolation step. To determine the optimal mixing conditions, three mixing speeds were tested on a KingFisher instrument: slow (baseline), medium, and fast. At the medium and fast mixing speeds, cell loss was significant (Figure 3). In addition, the remaining cells had reduced viability (data not shown). At the low mixing speed, isolation efficiency and cell viability were about the same as with manual cell isolation. Therefore, to obtain a high yield and high viability of isolated cells, the sample should be mixed gently during isolation.

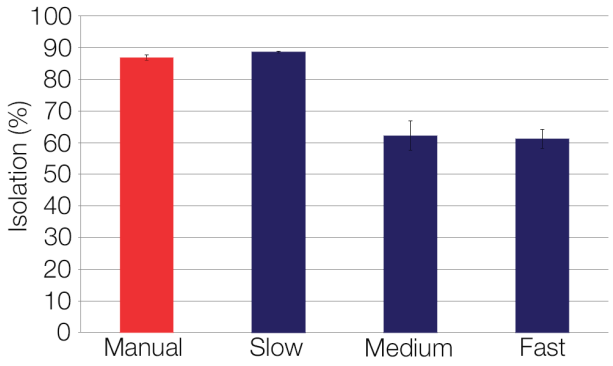

Figure 3. Mixing conditions for isolation of CD3-positive Jurkat cells. Jurkat cells were incubated with Dynabeads CD3 for 30 min at slow, medium, or fast mixing speeds. Cell isolation results were compared with those of the manual approach. Flow cytometry was used to estimate the isolation efficiency.

Optimization of bead capture after cell isolation

To maximize cell depletion, it is critical to remove all bead-bound cells. Here, we compared the depletion efficiency after bead-bound cells were captured one time (1x) to the efficiency after two (2x) and three (3x) cycles of bead capture. The results demonstrated that introducing one additional capture step resulted in depletion equal to or better than manual isolation (Figure 4).

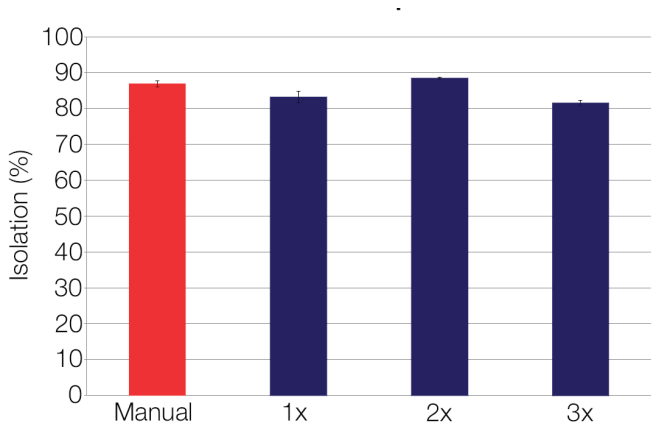

Figure 4. Bead depletion efficiency. Jurkat cells were incubated with Dynabeads CD3 for 30 min using a KingFisher instrument. The cells were collected 1x, 2x, or 3x, and isolation efficiency was compared with the manual approach. Flow cytometry was used to estimate the isolation efficiency.

Protocol performance on biologically relevant material

Protocol optimization was initially performed on cell lines to eliminate donor variation. An incubation time of 30 min with slow mixing followed by two cycles of bead capture resulted in isolation efficiency equal to or better than the manual approach. This optimized protocol was then used for isolation of target cells from more biologically relevant material such as PBMCs. Subclasses of T cells (CD3, CD4, CD8), monocytes (CD14), and B cells (CD19) from several blood donors were isolated using the optimized protocol on a KingFisher instrument. The performance was compared with the isolation efficiency obtained by the manual approach. Flow cytometry was used to estimate the isolation efficiency. The data effectively demonstrated that the isolation efficiency using the KingFisher instrument is about equivalent to a manual isolation approach for all the different cell types tested (Figure 5).

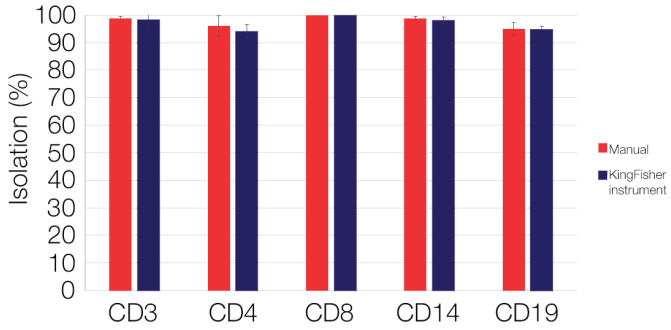

Figure 5. Efficiency of isolation of subclasses of T cells (CD3, CD4, CD8), monocytes (CD14), and B cells (CD19) from PBMCs. Dynabeads CD3, Dynabeads CD4, Dynabeads CD8, Dynabeads CD14, and Dynabeads CD19 were incubated with PBMCs for 30 min using a KingFisher instrument. Isolation efficiency with the KingFisher instrument was compared with the manual approach. Flow cytometry was used to estimate the isolation efficiency.

IP of target proteins from cells isolated on KingFisher instruments

Protein–protein interactions within cells are widely studied using IP or co-IP applications. Seamless integration of an automated cell isolation protocol with an automated IP or co-IP protocol will help ensure high reproducibility and significantly reduce overall protocol time and hands-on time. CD4-positive cells were isolated from PBMCs using Dynabeads CD4 on the Kingfisher Flex, Duo Prime, and Apex instruments. Flow cytometry analysis demonstrated isolation efficiency of 98% (Figure 6A). All three KingFisher instruments demonstrated similar isolation efficiency (Figure 6B). The Invitrogen™ FLoid™ Cell Imaging Station was used to confirm the presence of target cells on the beads after isolation, and the absence of cells after cell lysis (Figure 7). The cell lysate was prepared for protein isolation on the KingFisher instruments to capture CD81 using Dynabeads Protein G conjugated with CD81 antibody. Protein transfer and detection were performed using the iBlot 2 Gel Transfer Device and the iBind Western Device, respectively. The data clearly demonstrated the presence of the CD81 protein (Figure 8).

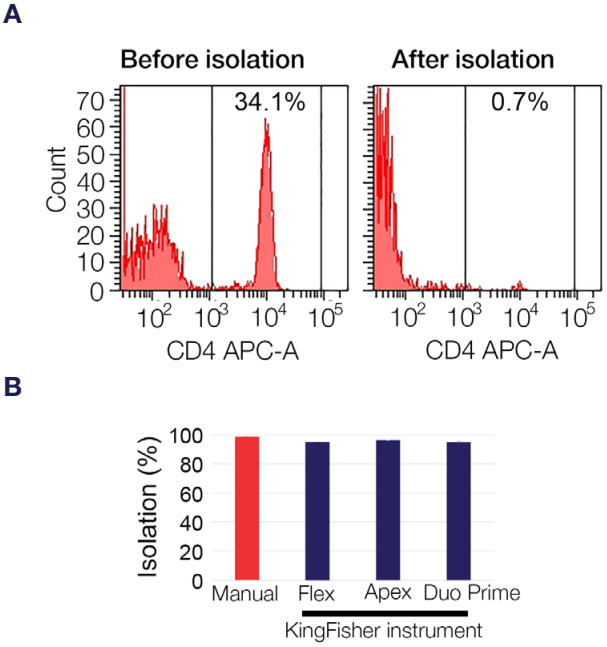

Figure 6. Efficiency of isolation of CD4-positive cells from PBMCs. **(A)** Dynabeads CD4 was incubated with PBMCs for 30 min using a KingFisher instrument. Depletion of CD4-positive cells was confirmed using flow cytometry. **(B)** CD4-positive cells were isolated using Dynabeads CD4 on the KingFisher Flex, Apex, or Duo Prime instrument and compared with the manual approach. Flow cytometry was used to estimate the isolation efficiency.

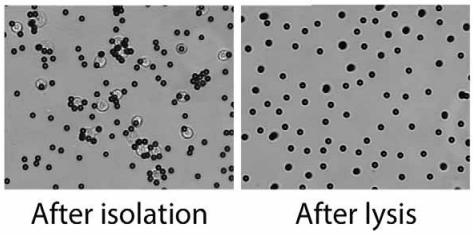

Figure 7. Lysis of CD4-positive cells after isolation. CD4-positive cells were isolated from PBMCs with Dynabeads CD4, followed by cell lysis. The FLoid Cell Imaging Station was used to visualize the bead-bound cells prior to lysis and confirm the absence of cells after lysis.

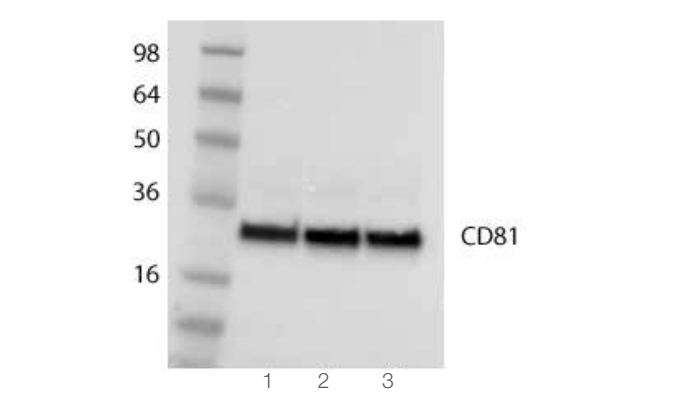

Figure 8. Western blot analysis of CD4-positive cells for CD81 marker. CD4-positive cells were isolated from PBMCs using the KingFisher instrument and Dynabeads CD4. Following cell lysis, CD81 was captured on the KingFisher instrument using Dynabeads Protein G conjugated with CD81 antibody. Samples were prepared for electrophoresis on Bolt gels. After protein separation, western blot transfer was performed using the iBlot 2 Gel Transfer Device, and immunolabeling was performed using the iBind Western Device. Lanes 1, 2, and 3 represent samples processed in parallel.

Conclusions

Here we have integrated an optimized, automated cell isolation approach with an optimized, automated IP method and simplified western blotting steps to complete the workflow within one day. In less than 7 hours, cells were efficiently isolated, followed by effective protein capture. In addition, manual processing was reduced for both protein transfer and labeling steps. With such an approach, many manual operations have been eliminated, and results can be evaluated within a typical workday.

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