Cell isolation

Automated T cell isolation featuring easy integration with downstream activation and immunoprecipitation

Keywords
CD3, CD4, CD8, T cells, Dynabeads, FlowComp, positive cell isolation, untouched isolation, T cell activation, Dynabeads Human T-Activator CD3/CD28, KingFisher Flex, Duo Prime, Apex, automation, flow cytometry, Attune, immunoprecipitation, western blot

Key findings

- Cell isolation using Dynabeads magnetic beads can be efficiently automated on the KingFisher purification system with high yield
- Automated cell isolation followed by automated immunoprecipitation using Dynabeads magnetic beads and subsequent western blot analysis can be performed within one working day
- Bead-based automated positive isolation of T cells gives activation profiles equivalent to those of manual positive or negative T cell isolation methods

Introduction

T cells are vital for the adaptive immune response, cell-mediated immunity, immune coordination and regulation, immunological memory, and immune tolerance. Their multifaceted functionality make them essential for maintaining a robust and balanced immune system. Thus, T cells remain at the forefront of research in areas such as cancer, autoimmune diseases, allergies, transplantation, and infectious diseases. Understanding the different functions of immune cells is of great importance, but T cells can be challenging to study without sufficient isolation and activation. Reliable methods to reduce sample complexity by isolating cells of interest or removing unwanted cells are therefore critical prior to downstream examination. Additionally, integrating an automatable workflow from cell activation to protein isolation and evaluation further simplifies this process.

A solution is offered by Invitrogen™ Dynabeads™ magnetic beads, which are nonporous, uniform, monodisperse superparamagnetic polystyrene beads that are widely used in various applications. The beads' uniformity and shape provide consistent properties and have been instrumental in providing high-performance isolation of cells, exosomes, proteins, viruses, nucleic acids, and other targets for more than 35 years.

Here we have developed and integrated a fully automatable cell isolation approach, along with an automated protein isolation method using Dynabeads magnetic beads and the Thermo Scientific™ KingFisher™ Flex, Duo Prime, or Apex system. This automated workflow can help users reduce their overall hands-on and experiment time from 2–3 days down to less than 7 hours (Figure 1). We also performed both positive and negative manual isolation of T cells, using Invitrogen™ Dynabeads™ FlowComp™ and Untouched™ kits, respectively, and compared them to automated positive isolation using Invitrogen™ Dynabeads™ FlowComp™ kits on the KingFisher instruments, to see whether the different isolation methods affected the downstream T cell activation process using Invitrogen™ Dynabeads™ Human T-Activator CD3/CD28 beads.

We demonstrate that an automated process of cell isolation and immunoprecipitation is easily executed within one workday, and that whichever cell isolation strategy is chosen prior to bead-based T cell activation the user can expect similar expression patterns and phenotypes of activated T cells.

Materials and methods

Isolation strategies

T cells were isolated from peripheral blood mononuclear cells (PBMCs) for downstream protein and cell analysis. Invitrogen™ Dynabeads™ CD3, CD4, and CD8 (Cat. No. 11151D, 11145D, and 11147D) were used for positive isolation prior to immunoprecipitation (IP), according to the user manuals.

Positive isolations to obtain bead-free cells prior to the activation process were performed using Invitrogen™ Dynabeads™ FlowComp™ CD3, CD4, and CD8 kits (Cat. No. 11365D, 11361D, and 11362D). The manual isolations were performed according to the user manuals. In short, the target cells were labeled with the supplied antibodies and washed, and then beads were added to bind the target cells. After a short incubation and wash to obtain cells of high purity, a release buffer was added to remove the beads from the target cells. The pure and bead-free T cells were used in the subsequent activation process. The manual negative isolation of T cells was performed using Invitrogen™ Dynabeads™ Untouched™ T cells, CD4 T cells, and CD8 T cells kits (Cat. No. 11344D, 11346D, and 11348D) according to the user manuals. In short, the supplied antibody mix was used to label all the unwanted cells in the PBMC sample, and Dynabeads magnetic beads were then added to bind the labeled cells. The unwanted bead-bound cells were depleted from the sample using a magnet, leaving behind the pure, viable naive T cells.

Protein and cell analysis

Isolated T cells were processed for flow cytometry to estimate the isolation efficiency. For IP and western blotting, isolated T cells were lysed using Thermo Scientific™ RIPA Lysis and Extraction Buffer (Cat. No. 89900). IP was performed using Invitrogen™ Dynabeads™ Protein G (Cat. No. 10003D) conjugated with Invitrogen™ CD81 Monoclonal Antibody (Cat. No. 10630D). Following SDS-PAGE, proteins were transferred to a PDVF membrane using the Invitrogen™ iBlot™ 2 Gel Transfer Device (Cat. No. IB21001). The Invitrogen™ iBind™ Solution Kit (Cat. No. SLF1020) was used for immunolabeling of CD81. HRP-conjugated TrueBlot™ ULTRA anti–mouse IgG (Rockland Immunochemicals, Cat. No. 188817-33) was used as the secondary antibody. Thermo Scientific™ SuperSignal™ West Dura Extended Duration Substrate (Cat. No. 37071) was used to develop the PVDF membranes after staining.

T cell activation

T cells were activated using Dynabeads Human T-Activator CD3/CD28 (Cat. No. 11131D) at a 1:1 bead:cell ratio according to the user manual. Cells were incubated with Dynabeads Human T-Activator CD3/CD28 for 8 days. Cell samples were taken on days 0, 6, and 8 and counted for the fold-change assessment.

Automation

Automated cell and protein isolation were performed on the KingFisher Flex, Duo Prime, or Apex system. Thermo Scientific™ BindIt™ Software (4.0) was used for the KingFisher Flex and Duo Prime systems, and Thermo Scientific™ BindIx™ Software was used for the KingFisher Apex system.

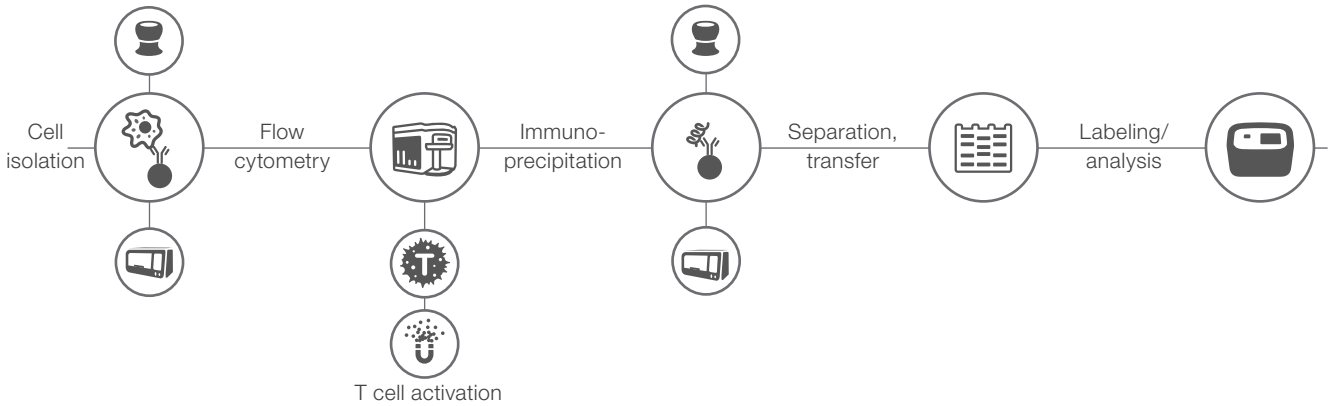

Figure 1. Automated cell and protein capture. Cell isolation and downstream applications are achievable within one working day.

Results

High purity of T cells regardless of isolation method

Positive isolation of T cells using Dynabeads FlowComp CD3, CD4, and CD8 kits, and negative isolation using Dynabeads Untouched T cells, CD4 T cells, and CD8 T cells kits, provided high purity (Figure 2). We did not find any difference between the manual approach and the automated positive isolation on the KingFisher system. Furthermore, we did not observe any phenotypic differences between the cells isolated by the two methods (Figure 3).

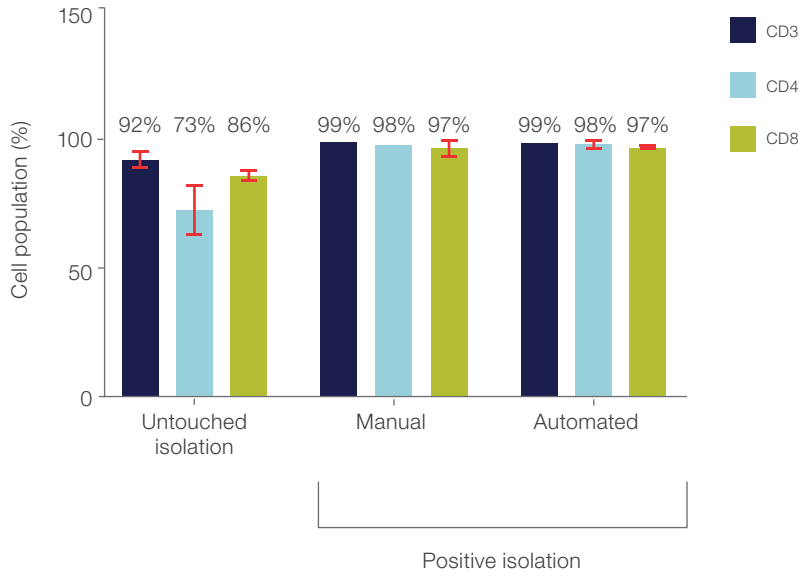

Figure 2. Efficiency of PBMC isolation using Dynabeads kits. Dynabeads kits were used to isolate T cell subsets by manual untouched isolation, manual positive isolation, and automated positive isolation on a KingFisher instrument. All isolations showed high purity of the target cells, with no difference found between manual and automated positive isolations.

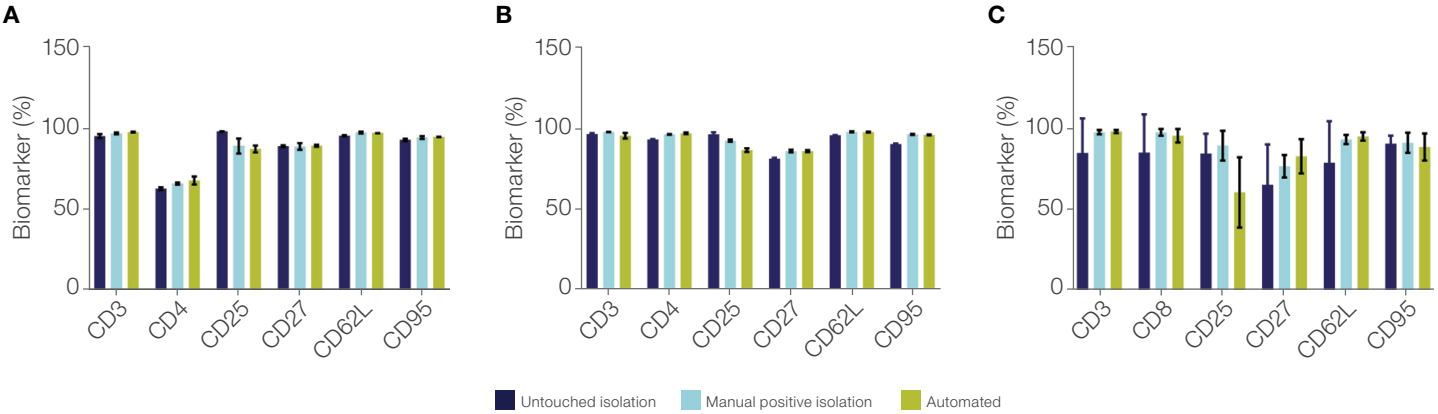

Figure 3. Isolation strategy for CD3⁺, CD4⁺, and CD8⁺ T cells does not affect phenotype. (A) CD3⁺, **(B)** CD4⁺, and **(C)** CD8⁺ T cells did not show any phenotypic differences between manual untouched isolation, manual positive isolation, and automated positive isolation on a KingFisher instrument.

Automated IP workflow done within a working day

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 can help ensure high reproducibility and significantly reduce overall protocol time and hands-on time.

The data clearly show excellent depletion after cell isolation, and the presence of the CD81 protein after IP (Figure 4). Performing all steps manually can take 2 days, while moving the process to automated cell isolation, IP, and western blot processing reduces the procedure to just 7 hours, enabling time savings, less manual error, and higher reproducibility between samples.

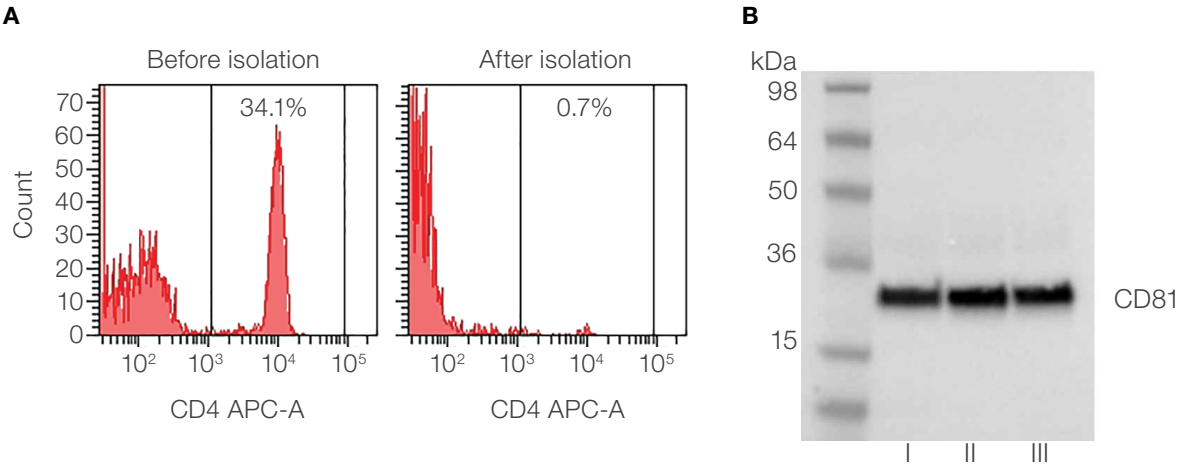

Figure 4. Cell isolation and IP protocols can be automated on a KingFisher instrument for reduced times-to-results. (A) Flow cytometry showing CD4 as an example biomarker before and after isolation of CD4⁺ T cells from a PBMC population. **(B)** Western blot showing the T cell costimulatory molecule CD81 after automated CD4⁺ T cell isolation followed by automated IP with Dynabeads Protein G and CD81 antibody.

T cell activation profile is independent of cell isolation strategy

When performing T cell activation and proliferation studies, it is important to know that the pre-isolated T cells have not been affected by the isolation method. Results for T cell expansion using Dynabeads Human T-Activator CD3/CD28 demonstrated excellent activation and proliferation of all T cell types, regardless of the isolation method used—untouched, manual positive, or automated positive (Figure 5).

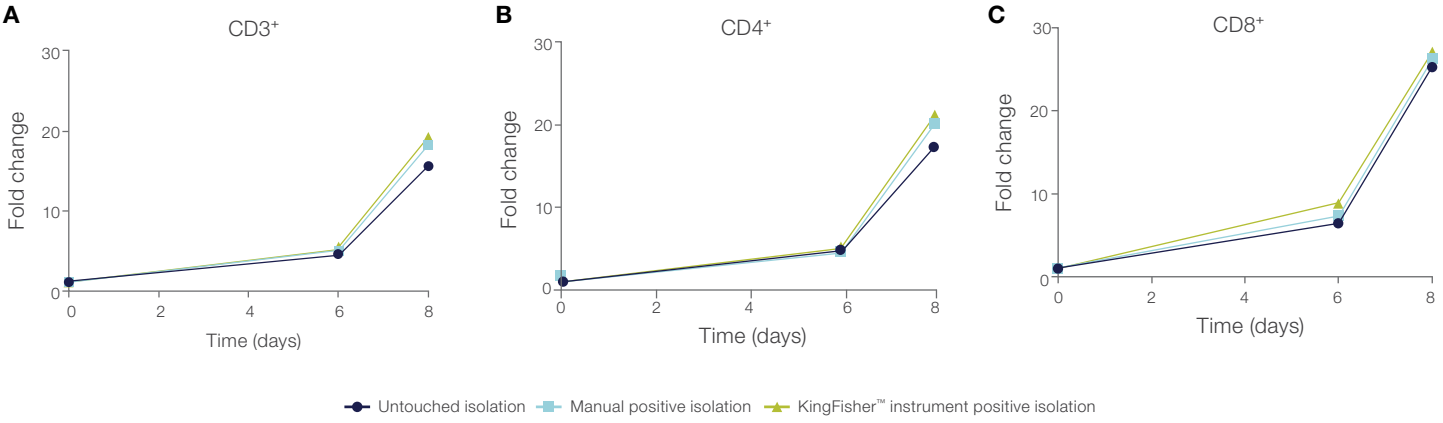

Figure 5. T cell expansion occurred at the same rate regardless of the isolation strategy used. (A) CD3⁺, **(B)** CD4⁺, and **(C)** CD8⁺ cells all proliferated similarly, independent of the isolation method (manual untouched, manual positive, and automated positive on a KingFisher instrument).

T cells were imaged over 8 days to show the characteristic changes that occur during the activation process. At 24 hours (Figure 6A), T cells are small and rounded. At 72 hours (Figure 6B) blasting, in which T cells become larger and more triangular in shape, is observed. Clustering of cells, which is required for cellular communication, is also seen. At 148 hours (Figure 6C) larger clusters have formed, with blasting still occurring. Finally on day 8 (Figure 6D), the T cell clusters begin to break apart, but they are still in the exponential growth phase.

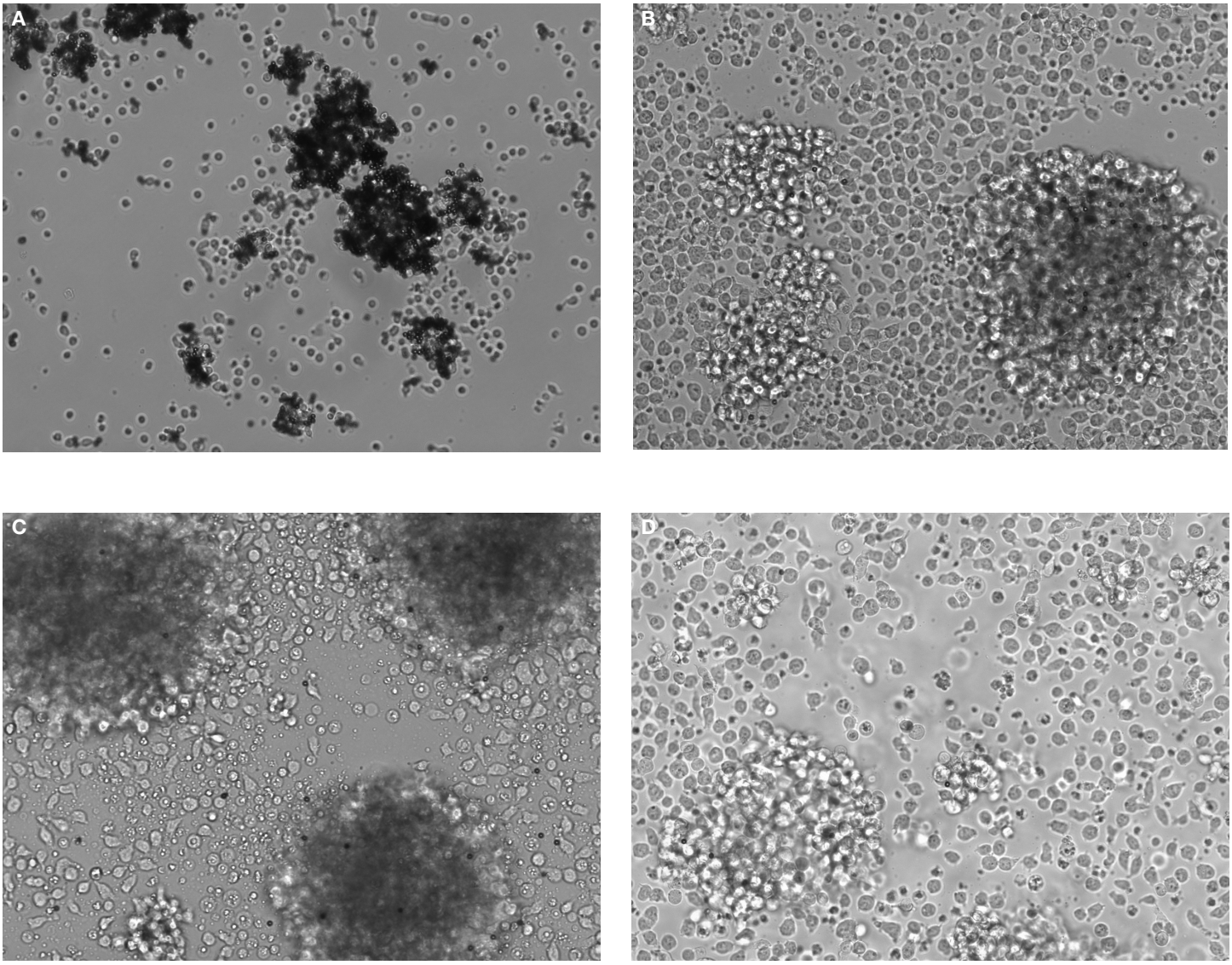

Figure 6. Light microscopy images of T cells incubated with Dynabeads Human T-Activator CD3/CD28 beads. (A) Cells incubated for 24 hours with beads begin to cluster and attach to T cells. **(B)** T cells incubated for 72 hours with beads begin blasting and clustering. **(C)** After 148 hours, cells have formed large clusters and continue to blast and proliferate. **(D)** Dynabeads magnetic beads have now fallen off T cells, and the cells form fewer clusters but continue to proliferate from activation signals.

Discussion

Here we have integrated an optimized automated cell isolation approach with optimized automated IP and western blotting approaches to complete a workflow within one day. In less than 7 hours, cells were efficiently isolated, followed by effective protein capture. In addition, both protein transfer and labeling were automated. With such an approach, many manual operations are eliminated and the results can be evaluated after less than 7 hours (Table 1).

We have shown three isolation strategies researchers can use, depending on their requirements, that do not interfere with cell viability or phenotype. T cell activation using Dynabeads Human T-Activator CD3/CD28 is robust regardless of the isolation strategy, and researchers can expect rapid activation and expansion of T cell populations without loss of integrity or phenotype.

Table 1. Comparison of hands-on time using manual and automated approaches for cell and protein isolation.

Protocol step	Manual	Automated
Cell isolation		
Capture target cells	20 min (incubation)	5 min (plate loading)
Wash 1	18 min (hands-on)	
Wash 2		
Wash 3		
Total hands-on time	18 min	5 min
Standard protocol time	38 min	36 min (script)
Immunoprecipitation		
Add antibody to Dynabeads beads	11 min (hands-on)	13 min (plate loading)
Incubate antibody-bead complex	10 min (incubation)	
Wash 1	6 min (hands-on)	
Capture target	10 min (incubation)	
Wash 2	18 min (hands-on)	
Wash 3		
Wash 4		
Elute target	10 min (incubation)	
Total hands-on time	35 min	13 min
Standard protocol time	45 min	45 min
Western blotting (1 blot)		
Preparation for western blotting	15 min (hands-on)	2 min (hands-on)
Standard protocol time	60 min	7 min
Immunolabeling (1 blot)		
Preparation for labeling	15 min (hands-on)	5 min (hands-on)
Standard protocol time	2 days	2.5 hr
Total workflow time		
Total hands-on time (cell and protein isolation)	83 min	25 min
Total standard protocol time	Approximately 2 days	<7 hr

Authors

Marc McGowan, Anette Kullmann, Berit Marie Reed, and
Ketil W. Pedersen, Thermo Fisher Scientific.

Ordering information

Product	Cat. No.
Dynabeads Untouched Human T Cells Kit	11344D
Dynabeads Untouched Human CD4 T Cells Kit	11346D
Dynabeads Untouched Human CD8 T Cells Kit	11348D
Dynabeads FlowComp Human CD3 Kit	11365D
Dynabeads FlowComp Human CD4 Kit	11361D
Dynabeads FlowComp Human CD8 Kit	11362D
Dynabeads CD3	11151D
Dynabeads CD4	11145D
Dynabeads CD8	11147D
Dynabeads Human T-Activator CD3/CD28	11131D
Dynabeads Protein G for Immunoprecipitation	10003D
KingFisher Flex Purification System	5400630
KingFisher Duo Prime Purification System	5400110
KingFisher Apex Purification System	5400910
RIPA Lysis and Extraction Buffer	89900
CD81 Monoclonal Antibody (M38)	10630D
iBlot 2 Gel Transfer Device	IB21001
iBind Western Device	SLF1000
SuperSignal West Dura Extended Duration Substrate	37071
Optional products	
DynaGreen Protein A Magnetic Beads	80101G
DynaGreen Protein A/G Magnetic Beads	80104G
DynaGreen CaptureSelect Anti-IgG-Fc (Multi-Species) Magnetic Beads	80107G

Learn more at **thermofisher.com/cellisolation**