

# Avoid Artifacts: Isolate Pure and Functional Monocytes from PBMC, Buffy Coat or Whole Blood using Dynabeads® FlowComp™

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## Aim

Develop a protocol for positive isolation of bead-free monocytes.

## Materials and Methods

Dynabeads® FlowComp™ technology isolates human monocytes where, in the first step, FlowComp™ Human CD14 Antibody conjugated to a modified biotin binds to the monocyte. In the second step CD14+ monocytes, that have bound the specific antibodies, are captured by the Dynabeads® which are conjugated to modified streptavidin. In the last step, beads are gently removed from the cells by adding a release buffer which have higher affinity for the streptavidin conjugated Dynabeads.

## Results

Freshly isolated monocytes using Dynabeads® FlowComp™ Human CD14 have excellent purity (>98%) (Fig 1). The kit allows for isolation from PBMC, or directly from buffy coat and whole blood. Isolation from buffy coat and whole blood yields cells with equally high purity, recovery and viability as when starting from PBMC (Fig 2a,b). In addition, the protocol is approximately 1.5 h shorter and cost is reduced (Fig 2c). After isolation the bead-free monocytes were tested in functional assays. First, monocytes cultured in medium supplemented with GM-CSF and IL-4 differentiate into monocyte-derived DC, up-regulating HLA-DR and CD86 expression and losing CD14 expression turning them into efficient antigen-presenting cells (Fig 3). Upon LPS stimulation, isolated monocytes start producing TNF- $\alpha$  as demonstrated by intracellular staining (Fig 4).

## Discussion

Dynabeads® FlowComp™ Human CD14 can be used to isolate monocytes from PBMC, and directly from whole blood or buffy coat with no extra reagents required. Cells isolated directly from buffy coat or whole blood requires less manipulation of your cells, thus you obtain a higher viability of cells in a more ex vivo state. Starting with whole blood or buffy coat also saves time and money because there is no need for PBMC preparation. Dynabeads® FlowComp™ Human CD14 allows for gentle isolation of bead-free monocytes ready to be used in subsequent bioassays.

## Ordering Info

Dynabeads® FlowComp™ Human CD14	113.67D
Anti-human CD14 APC (clone Tuk4)	MHCD1405
Anti-human CD4 Alexa-488	MHCD0420
Sytox-Blue	S34857
DynaMag™-15	123.01D
DynaMag™-50	123.02D
HulaMixer™ Sample Mixer	159.20D

## Isolation of bead-free CD14+ monocytes using Dynabeads® FlowComp™

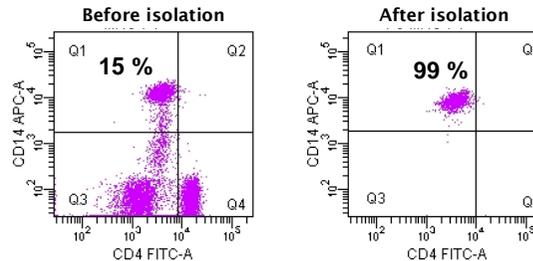


Fig 1: Dynabeads® FlowComp™ Human CD14 was used to isolate bead-free monocytes from prepared PBMC with high purity.

## Benefits when isolating directly from Buffy Coat or Whole Blood

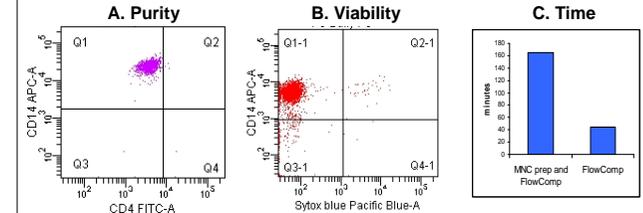


Fig 2: Purity and viability (A, B) of monocytes isolated directly from whole blood or buffy coat is of same high quality as when obtained by isolating from PBMC. However, when isolating from whole blood/buffy coat, no time, money or unnecessary cell loss is wasted on PBMC preparation (C), giving the benefits of a faster protocol without sacrificing the purity or viability.

## Highly pure and bead-free monocytes show intact functional responses in subsequent experiments

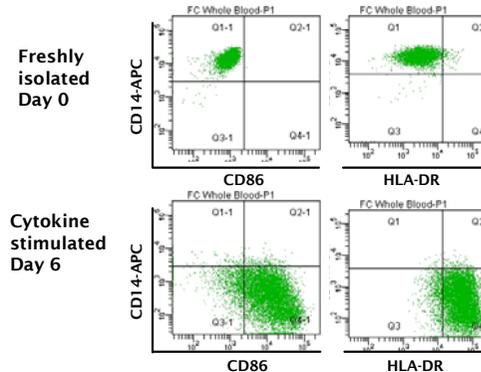
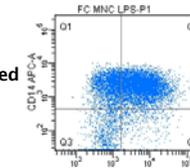


Fig 3: Isolated monocytes differentiate into monocyte-derived DC when cultured with IL-4 and GM-CSF. Freshly isolated monocytes were cultured in medium supplemented with IL-4 (500 ng/ml) and GM-CSF (800 ng/ml) for 6 days. Flow cytometric analysis was performed at day 0 and day 6. Results demonstrate that CD14 is lost during culture and cells up-regulate HLA-DR and CD86. More than 60% recovery of monocyte-derived DC is observed. Picture shows monocytes isolated from whole blood, and similar results was observed when starting with PBMC or buffy coat.

LPS stimulated 4h



Freshly isolated 0h

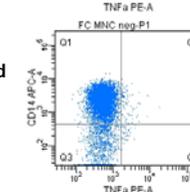


Fig 4: Isolated monocytes produce TNF- $\alpha$  in response to LPS stimulation. Freshly isolated bead-free monocytes were stimulated with LPS for 4h prior to intracellular staining with TNF- $\alpha$ . Stimulated cells were incubated with Brefeldin A. Non-stimulated cells is shown in the lower diagram.

## CONCLUSIONS

- @ Dynabeads® FlowComp™ Human CD14 isolates bead-free monocytes with excellent purity
- @ Isolation directly from whole blood or buffy coat saves time, reduces costs and increases viability
- @ Isolation with Dynabeads® FlowComp™ technology ensures bead-free monocytes with intact functional responses in subsequent experiments