HUMANZYME
HUMAN PROTEINS FROM HUMAN CELLS

PRODUCT DATA SHEET
G4 DC Generation Kit

HUMANKINE™
G4 Dendritic Cell Generation Kit

Product Description

- Produces high quality dendritic cells
- Yields that are equal to or better than standard protocols
- No medium change or addition required
- No cytokine aliquoting required
- Xeno and Animal-component free
- Human cell expressed growth factors included

HumanKine™ G4 Dendritic Cell (DC) Generation Kit has been developed for the growth and generation of human dendritic cells (DCs). Using HumanKine G4 DC medium, human dendritic cells can be produced from peripheral blood mononuclear cells WITHOUT MEDIUM CHANGE and used either as immature DCs or further cultivated into mature DCs.

Protocol in Brief
Reconstitute Cytokine Cocktail
Add Cocktail to Basal Medium
Suspend monocytes in complete medium
Incubate 7 days
DO NOT CHANGE MEDIUM
DO NOT ADD MEDIUM

Specifications

Kit Contents: G4 DC Basal Medium
Growth Factor Cocktail
Protocol
Kit includes all necessary medium and reagents (except beta-mercaptoethanol and Fetal Calf Serum)

Pack Size: 100 mL HZ-9101 and
500 mL HZ-9501
NOTE: A 10 mL culture requires only 10 mL medium. (plating 3-5 x10^5 cells/ml) No medium addition or change is required.

Quality Confirmation

A certificate of analysis is available on request.

Storage and Stability

G4 DC Basal Medium: Store at 4°C, protect from light. Medium is stable for six months from date of production.
G4 DC Cocktail (Lyophilized): Store at -80°C. Cocktail is stable for twelve months from date of receipt.

Complete medium (G4 DC Cocktail added to G4 DC Basal Medium): Can be stored at 4°C for up to 30 days in the dark.

Adding the Growth Factor Cocktail to the G4 DC Basal Medium produces G4 Dendritic Cell Generation Medium requiring no medium addition or change during the incubation period.

The resulting cells are monocyte-derived immature DCs that are highly phagocytic with the immature DC surface expression phenotype ($CD14^{neg}$, $CD83^{neg}$, $CD80^{low}$, $CD86^{low}$, MHC I^{int}, and MHC^{int}) and can migrate in response to CCR5 agonist (RANTES), but not to CCR7 agonists (ELC/SLC).

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HUMANKINE™ MEDIUM Kit

G4 Dendritic Cell Generation Kit Protocol

HumanKine™ G4 Dendritic Cell (DC) Generation Kit has been developed for the growth and generation of human dendritic cells (DCs). Using HumanKine G4 DC medium, human dendritic cells can be produced from peripheral blood mononuclear cells WITHOUT MEDIUM CHANGE and used either as immature DCs or further cultivated into mature DCs. A certificate of analysis is available on request.

Intended Use
For research use only. Not intended for human or animal diagnostic or therapeutic uses.

Storage
G4 DC Basal Medium: Store at 4°C, protect from light.
G4 DC Cocktail (Lyophilized): Store at -80°C.
Complete medium (G4 DC Cocktail added to G4 DC Basal Medium): Can be stored at 4°C for up to 30 days in the dark. It is recommended to aliquot the complete medium into required working amounts and to avoid exposing the complete medium to 37°C multiple times.

Shelf Life
G4 DC Basal Medium: Six months from date of production
G4 DC Cocktail (Lyophilized): Twelve months from date of receipt

A. Complete Medium Preparation

G4 DC Basal Medium is composed of RPMI 1640 that has been supplemented with L-Glutamine, 25mM HEPES, 100 units Penicillin and 50µg/mL Streptomycin. G4 DC complete medium requires final concentration of 10% Fetal Calf Serum, 50µM beta-mercaptoethanol, and G4 DC Cocktail added to the G4 DC Basal Medium. Complete medium is stable for 30 days when stored at 4°C in the dark.

1. For 500mL (100mL) complete medium, aseptically add 50mL (10mL) Fetal Calf Serum to G4 DC Basal Medium, HZ-9501-m (HZ-9101-m).

2. Aseptically add 5mL (1mL) of 5mM beta-mercaptoethanol to 500mL (100mL) medium.

3. Briefly centrifuge the G4 DC Cocktail vial prior to opening to draw contents to the bottom of the vial. Aseptically resuspend G4 DC Cocktail, HZ-9501-c (HZ-9101-c) in 0.5mL (0.1mL) sterile 1x PBS. Transfer the liquid cocktail to 500mL (100mL) of medium, rinse tube with complete medium and transfer liquid to 500mL (100mL) of medium.

B. Monocyte Preparation

1. Isolate peripheral blood mononuclear cells from blood by Ficoll gradient centrifugation.

2. Isolate monocytes from peripheral blood mononuclear cells by using either Percoll gradient centrifugation (Monocyte purity ~90%) or using CD14 monocyte purification kit available from MiltenyiBiotec (Monocyte purity ~ 95–98%).

C. Immature Dendritic Cell Generation

This protocol is designed for use with product number HZ- 9501 (HZ-9101), HUMANKINE™ G4 Dendritic Cell Generation Kit. In this protocol, HumanZyme GM-CSF^{HuXp} and IL-4^{HuXp} are used to generate human monocyte-derived DCs. This protocol DOES NOT REQUIRE MEDIUM CHANGE OR MEDIUM ADDITION over the 7 day incubation period.

1. Suspend monocytes in prewarmed (37°C) G4 DC complete medium at 3-5x10^{5}cells/ml.

2. Culture the monocyte suspension in a humidified incubator with an atmosphere containing 5% CO_2 for 7 days. DO NOT ADD ADDITIONAL MEDIUM OR CHANGE THE MEDIUM.

3. Harvest mildly adherent and floating cells by centrifuging at 400 x g for 5 minutes at room temperature. The resulting cells are monocyte-derived immature DCs that are highly phagocytic with the immature DC surface expression phenotype (CD14^{neg}, CD83^{neg}, CD80^{low}, CD86^{low}, MHC I^{int}, and MHC^{int}) and can migrate in response to CCR5 agonist (RANTES), but not to CCR7 agonists (ELC/SLC).

D. Maturation of Immature Dendritic Cells

1. Suspend immature DCs at 5x10^{5}cells/ml in fresh complete G4 DC medium.

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2. Culture in the presence of LPS (0.1-1 µg/ml) or CD40 ligand (10-20 µg/ml) in a humidified incubator with an atmosphere containing 5% CO_2 for 48 hours.

3. Mature DCs downregulate their phagocytic capacity, display surface marker phenotype typical of mature DCs ($CD14^{neg}$, $CD83^{pos}$, $CD80^{int}$, $CD86i^{nt}$, MHC I^{high}, and MHC^{high}) and migrate in response to CCR7 agonists (ELC/SLC), but not to CCR5 agonist (RANTES).

4. Alternatively, maturation can be induced by adding 100U/ml TNF-alpha. (Cat. No. HZ-1014)

References:
Romani et al.1994. J Exp Med 180: 83-93.
Yang D. et al. J Immunol. 2004. 173 : 6134-6142
Tedder TF and Jansen PJ. 1997. Current Protocol in Immunology. 7.32.1-7.32.6

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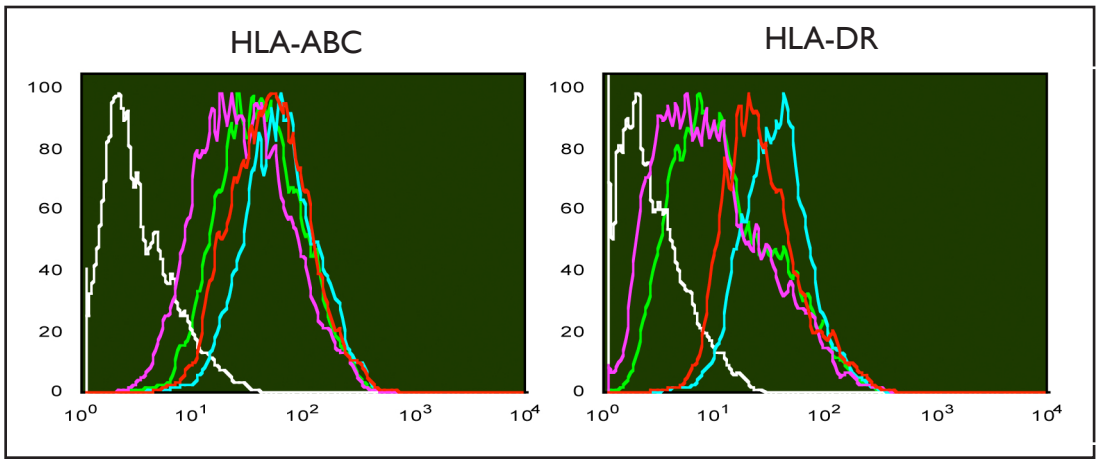

Fig. 1. Expression of HLA-ABC and HLA-DR by DCs.

White: isotype-matched control
Green: HumanKine G4 DCs without medium change and without LPS treatment
Blue: HumanKine G4 DCs without medium change and with LPS treatment
Pink: E. coli G4 DCs with medium change andwithout LPS treatment
Red: E coli G4 DCs with medium change and with LPS treatment.

The data show that HumanKine™ G4 DCs without medium replacement are similar or even better than DCs differentiated in the presence of E. coli expressed cytokines with standard G4 protocol requiring medium change. More comprehensive data can be found at www.humanzyme.com/products.

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