Product Datasheet

DC Differentiation and Maturation Monitoring Assay Kit
NBP2-29607

Unit Size: 1 Kit

Store at 4C in the dark.

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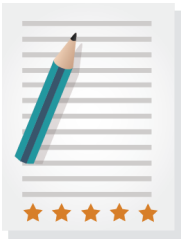

NBP2-29607

DC Differentiation and Maturation Monitoring Assay Kit

Product Information	
Unit Size	1 Kit
Concentration	Concentration is not relevant for this product. Please see the protocols for proper use of this product.
Storage	Store at 4C in the dark.

Product Description	
Description	DC Differentiation and Maturation Monitoring Kit can be used for: Qualitative analysis of DC preparation using only a small number of cells in multicolor flow cytometry. Monitoring changes in phenotype of DCs generated by modified protocols which include change of cytokine or maturation agents. Useful tips to set-up flow staining test: 1. In DC preparations, where immature (GMCSF+IL-4) and mature DC (GMCSF+IL-4 + maturation agent) are compared, set up two separate tubes for each sample. 2. In a 3 color test, when differentiation & maturation status is analyzed, use the components of the kit with no interference of conjugate in the same channel of analysis. 3. Unstained and single color control tubes are useful for instrument set-up and compensation. 4. First time users are advised to include matched isotype controls with same conjugates as testing antibody, in staining protocols. Note: isotype control antibodies are not provided in this kit. 5. Read the application and testing protocol before starting the experiment and contact our technical team for any additional clarification.
Species	Human
Kit Components	CD83 HB15e PE Mouse IgG1 k Human 25Tests, CD86 IT2.2 Alexa Fluor 647 Mouse IgG2b k Human 25Tests, HLA-DR L243 PE Mouse IgG2a k Human 25Tests, HLA-DR L243 PerCP-Cy5.5 Mouse IgG2ak Human 25Tests, CD14 RPA-M1 FITC Mouse IgG1k Human 25Tests

Product Application Details	
Applications	Flow Cytometry
Recommended Dilutions	Flow Cytometry

Procedures

Product Handling Guide (NBP2-29607)
Typical Protocol to generate DC from peripheral blood monocytes:

1. Generate DC following conventional protocol, typically by culturing monocytes with both GMCSF and IL-4, followed by maturation with TNF-alpha.

2. Enrich monocytes from total PBMC by plate adherence method. Briefly, layer PBMC (1-2x10^6/ml) in 6-well plates and allow monocytes to adhere by keeping the culture at 37C for 2h. After 2 hours, remove non-adherent cells. Rinse wells with RPMI -10 complete medium to remove the non-adherent cells completely.
Note: DCD&MM kit works also with DC generated from monocytes enriched by alternate protocols including, magnetic bead separation or RosetteSep.

3. Culture monocytes in 3 ml of complete medium containing recombinant human GMCSF (25 ng/ml) and IL-4 (20 ng/ml). Replenish cultures with fresh medium containing same cytokines.
Note: Concentrations can be standardized, based on suppliers' recommendations.

4. On Day 5, treat cultures with maturation agent (typically TNF at 25-50ng/ml) along with GMCSF & IL-4. As needed, cultures can be given a second dose of maturation signal and harvested for analysis on the following day.
Note: Other maturation agents such as LPS and other TLR ligands or a cocktail of inflammatory agents, can also be used to induce maturation.

5. DC cultures, when observed by microscopy appear in clusters and show typical dendrite morphology.

6. In this short term protocol, mature DCs are ready for phenotype and functional analysis on Day 6 to Day 8.

Note: It is good practice to continue to feed cultures with fresh medium and cytokines until harvest and analysis.

Assay Instructional Manual (NBP2-29607)
Protocol for immune-phenotype analysis of DC:

1. Wash DC from cultures twice with 1x FACS buffer and suspend in the same buffer.

2. Take typically 0.5-1x10^6 cells in 100ul of buffer for staining.

3. Add 5ul each of CD14 FITC, CD86 AF647, HLA-DR PerCP-Cy5.5 and 20ul of CD83 PE antibody, mix well.

4. In alternate tubes, use equal number of cells for unstained and isotype (not provided but details are given) controls.

5. Single color tubes are advised for each analysis.

6. Incubate all tubes on ice for 20 minutes, in the dark, for staining.

7. Wash cells by adding 2ml of 1x FACS buffer, by centrifugation (1200 RPM, 10 min). Repeat twice. Gently aspirate or decant the buffer after each wash and vortex tube at a very low speed, to break the pellet of cells.

8. After final wash, suspend in suitable amount (200-300ul) of staining buffer and analyze the samples.

9. Add PPI as needed to gate live cells for analysis (we typically use 5ul of 50ug/ml stock and incubate cells on ice for a quick/same day analysis).

MSDS (NBP2-29607)
Hazard Information
Chemical Name: Sodium Azide
Chemical Formula: NaN3
CAS Number: 26628-22-8
EEC-No: 247-852-1

Hazard Identification
Very toxic if swallowed. Contact with acids liberates very toxic gas.

First Aid Measures
Eye Contact: Irrigate thoroughly with water for at least 15 minutes. Seek medical advice.
Skin Contact: Wash skin thoroughly with soap and water for at least 15 minutes. Remove contaminated clothing and wash before re-use. In severe cases, obtain medical attention.
Inhalation: Remove from exposure, rest and keep warm. In severe cases, seek medical advice.
Ingestion: Wash out mouth thoroughly with water and give plenty of water to drink. Seek medical advice.

Accidental Release Measures
Wear appropriate protective clothing. Inform others to keep a safe distance. Spread soda ash liberally over spillage. If local regulations permit, mop up cautiously with plenty of water and run to waste, diluting greatly with running water. Otherwise transfer to container and arrange removal by disposal company. Wash site of spillage thoroughly with water.

Handling and Storage
Handling: Avoid prolonged contact with copper or lead, especially in drainage systems or mercury and other heavy metals which may result in the formation of explosive azides. Under no circumstances eat, drink or smoke while handling this material. Wash hands thoroughly after working with this material. Contaminated clothing should be removed and washed before re-use.
Exposure Controls / Personal Protection
Respirator: Dust respirator
Ventilation: Extraction hood
Gloves: Rubber or plastic
Eye Protection: Lab goggles or face shield
Other Precautions: Plastic apron, sleeves, boots - if handling large quantities.

Physical and Chemical Properties
Form: Liquid
Color: Colorless
Odor: Odorless
Melting Point: No data available
Boiling Temperature: No data available
Density: No data available
Vapor Pressure: No data available
Solubility in Water: Very soluble
Flash Point: No data available
Explosion limits: No data available
Ignition Temperature: No data available

Stability and Reactivity
Stable unless heated.
Slow reaction at ambient temperature unless water contains dissolved carbon dioxide. Decomposes violently with chromyl chloride. Contact with acids liberates highly toxic gas: forms readily detonable salts with many materials, particularly heavy metals.

Toxicological Information
After ingestion, irritation of mucous membranes in the mouth, pharynx, esophagus and gastrointestinal tract. Danger of skin absorption.

Disposal Considerations
Chemical residues are generally classified as special waste, and as such covered by regulations which vary according to location. Contact your local waste disposal authority for advice, or pass to chemical disposal company. Rinse out empty containers thoroughly before disposal.

Other Information
The information contained in this material safety datasheet is believed to be accurate but it is the responsibility of the user to determine the applicability of these data to the formulation of necessary safety precautions. NOVUS shall not

be held responsible for any damage resulting from the use of the above product or the information contained in this material safety data sheet.

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Limitations
This product is for research use only and is not approved for use in humans or in clinical diagnosis. Kits are guaranteed for 6 months from date of receipt.

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