DENDRITICS
INNOVATIVE TOOLS AROUND DENDRITIC CELLS

Animal Blood B Booster®
Kit
(DDXK-AnBBB)

This kit is an extension of the Human Blood B Booster Kit (DDXK-HuBBB) to animal species. Used in combination with the murine myeloma SP2/0 cell line, the Animal Blood B Booster kit (DDXK-AnBBB) is intended for immortalization of B lymphocytes isolated from a wide range of animal species (validated in horse, rabbit, sheep, rat, dog).

Background:

Dendritics routinely uses the hybridoma technology described by Köhler and Milstein, where the freshly isolated splenocytes of immunized mice, are fused with the Ig non secreting murine myeloma SP2/0 cell line to immortalize Ig secreting B lymphocytes. The resulting repertoire is generally limited either by the low immunogenicity of the antigen, or by the natural immunity of the mouse.

Because the Blood B Booster system (DDXK-HuBBB) was demonstrated as being powerful for human B cells, we attempted to improve the efficiency of the hybridoma technology by introducing an activation step before the fusion in an attempt to give access to a broader repertoire. Briefly, after the immunization program, freshly isolated splenocytes were separately stimulated according to two stimulation systems based on B cell activation through CD40 triggering. We have assumed that the inter-species homology of the CD40 antigen will allow non human B cell activation by a monoclonal antibody raised against human CD40. After the demonstration that the introduction of the stimulation step before fusion significantly increased the clonal diversity of the immortalized murine B cell populations, this process was successfully extended to other animal species starting from peripheral blood samples *(Razanajaona-Doll D et al, AAI 98th annual meeting, Immunology 2011™, San Francisco, CA, USA).*

The kit AnBBB® was packaged so as to make it very easy to handle for the scientists ; the kit AnBBB® contains all the reagents needed to obtain around 3.10^3 independent B cell clones from 6.10^7 splenocytes or PBMC using six 96 wells culture plates for the stimulations and ten 96 wells plates for the fusion step. The resulting cells are available for a wide range of studies including genomic, transcriptomic and proteomic fields.

Kit contents:

Optimal efficiency of the kit relies on the immediate storage of the reagents as recommended upon receipt.

color	vial n°	designation	volume	storage
blue	1	support 1	freeze-dried	-20°C
	2	mAb1	200µl	-20°C
	3	mAb2	200µl	-20°C
pink	4	support2	freeze-dried	-20°C
	5	mAb3	200µl	-20°C
booster reagent			67ml	-20°C

Not supplied reagents and materials:

D-MEM/F12 (Invitrogen # 31331-028)
L-Glutamine 200mM (Invitrogen, # 25030-024))
Flat bottom 96w plates BD Falcon (Dutscher, # 353072)
Reagents and materials for PBMC isolation from peripheral blood
Sp2/0 *murine* myeloma cell line *(ATCC #CRL 1581)*
Reagents and materials for hybridoma technology

WARNING

*Usage of ACD (acid-citrate-dextrose) tubes (yellow top) is recommended for blood collection.
*The stimulation could be affected (lowered and/or delayed) for blood samples containing more than 15% of $CD14^+$ monocytes.
*The efficiency of the process can be significantly affected for blood samples containing less than 2% of $CD19^+$ B lymphocytes.
*For the generation of heterohybridoma, usage of the SP2/0-Azaserine-PEG system is recommended (80% of Ig secreting cells)

Experimental procedure:

Prerequisite: obtain splenocytes or PBMC from naturally or experimentally immunized animals at Day 0.

The different vials are regrouped by color according to the stimulation scheme:

Stimulation 1: blue vials
Stimulation 2: pink vials

The Booster reagent is used to prepare the complete culture medium.

Day-1:

Prepare complete medium:
-500ml DMEMF12
-20ml L-Glutamine (8mM final concentration)
-67ml Booster reagent

The complete medium can be stored at 4°C for several weeks.

Reconstitute vial1 with 2ml of complete medium

MIX A

- 30ml of complete medium
- Reconstituted vial1 (2ml)
- Vial2 (200µl)

DENDRITICS SAS 60, AVENUE ROCKEFELLER 69008 Lyon -France. Tel +33 (0)4.72.71.74.03 E-mail : contact@dendritics.net
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- Vial3 (200µl)

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Distribute 100µl/well of **MIX A** in three 96 wells culture plates.
Incubate overnight at 37°C, under 5 %CO_2

Day 0:
Reconstitute vial 4 with 2ml of complete medium

MIX B
- 30ml of complete medium
- Reconstituted vial4 (2ml)
- Vial5 (200µl)

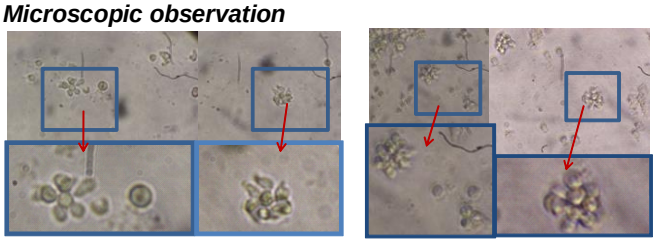
Distribute 100µl/well of **MIX B** in three 96 wells culture plates.
Incubate at 37°C, under 5 %CO_2

Cell preparation:
-Isolate splenocytes or peripheral blood mononuclear cells according to standard procedures.
-Resuspend cells in 50ml of PBS
-Spin at 1100 rpm for 20 min, RT
-Discard the supernatant and resuspend the pellet in complete medium
-Adjust cell concentration: 6.10^7 PBMC in 60ml of complete medium

Take out the culture plates from the incubator and distribute 100µl/well of the cell suspension in the six culture plates (3 plates for stimulation 1 and 3 plates for stimulation 2).

Incubate 3-5 days at 37°C, under 5 %CO_2

Microscopic observation



Microscopic observation of canine lymphocyte activated through stimulation 1 (left panel) and stimulation 2 (right panel) for 2 days

An increasing number of cellular patches constituted of hairy cells should be observed.

After 3-5 days of activation, cells are harvested, pooled and fused with SP2/0 myeloma cell line according to the standard procedure.

WORKFLOW

DAY -1

- Distribute 100µL/well of **MIX A**
- Incubate culture plates at 37 C, 5%CO_2

DAY 0

- Distribute 100µl/well of **MIX B**
- Incubate culture plates at 37 C, 5% CO_2
- Isolate splenocytes or PBMC from the animal donor
- Resuspend 6.10^7 cells in 60 ml of complete medium
- Take out all the culture plates from the incubator
- Distribute at 100µl/well of the cell suspension
- Incubate culture plates for 4 days at 37 C, 5% CO_2

DAY 1-3

- Proliferating hairy cells should be observed under microscope

DAY 4

- Harvest and pool the cells from the stimulations plates
- Proceed to the fusion using SP2/0 cell lines according your usual procedures

References:

1-Growing human B lymphocytes in the CD40 system.
Banchereau J, Rousset F. *Nature. 1991 Oct 17; 353 (6345):678-9.*
2-Polyclonal Ig production after Epstein-Barr virus infection of human lymphocytes in vitro.
Rosén A, et al, *Nature. 1977 May 5;267(5606):52-4.*
3-EB virus-induced B lymphocyte cell lines producing specific antibody.
Steinitz M, et al., *Nature. 1977 Sep 29;269(5627):420-2.*
4-Monoclonal antibody production by EBV transformation of B cells.
WO/2004/076677. 2004 Sept. 10. *Lanzavecchia A, inventors*;
5- A novel method for making human monoclonal antibodies.
Fraussen J, et al., *J Autoimmun. 2010 Sep;35(2):130-4.*
6-Generation of a high efficiency peripheral B lymphocyte immortalization tool. Razanajaona-Doll D et al, *AAI 98th Annual meeting, Immunology 2011™, San Francisco, CA, USA*

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Material Safety Data Sheet (Booster reagent)

Section 1-Chemical product

Name: Booster reagent
Synonym: serum substitute for mammalian cell culture
Company: Dendritics SAS
60 avenue Rockefeller
69008-Lyon, France
contact@dendritics.net
Tel: +33472717403

Section 2- Composition, physical and chemical properties

This product contains no substance which at their given concentration are considered to be hazardous to health
Physical state: delivered under liquid form
Odor: odorless
Color: pink solution

Section 3- Hazards identification

Hazard status: no specific hazard
Emergency overview: USE WITH CARE
Follow good industrial hygiene practice

Section 4- First aid measures

Skin contact: wash off immediately with plenty of water
Eye contact: rinse thoroughly with plenty of water, also under eyelids
Ingestion: never give anything by mouth to an unconscious person
Inhalation: move to fresh air
Notes to physician: treat symptomatically

Section 5- Fire-fighting measures

Suitable extinguishing media: use an extinguishing agent suitable for the surrounding fire
Special protective equipment: wear self-contained breathing apparatus and protective suit **for firefighters**

Section 6- Accidental release measures

Personal precautions: immediately contact emergency personnel. Keep unnecessary personnel away. Use suitable personal protective equipment
Methods for cleaning up if emergency personnel are unavailable contain spilled material. For small spills, add absorbent (soil may be used in the absence of other suitable materials), scoop up material and place in a sealable, liquid-proof container for disposal. For large spills, dike spilled material or otherwise contains material to ensure runoff does not reach a waterway. Place spilled material in an appropriate container for disposal.

Section 7- Handling and storage

Handling: wash thoroughly after handling
Storage: keep in properly labeled containers; keep container in a cool, well-ventilated area. Storage temperature 2-8°C (36-46°F)

Section 8- Exposure controls/personal protection

Exposure limits
Engineering measures: ensure adequate ventilation, especially in confined areas
Personal protective equipment
Respiratory protection: a respirator is not needed under normal and intended conditions of product use.
Hand protection: disposable vinyl gloves
Eye protection: safety glasses
Skin and body protection: lab coat
Hygiene measures: handle in accordance with good industrial hygiene and safety practice
Environmental exposure controls: prevent product from entering drains

Section 9- Stability and reactivity

Stability: stable
Materials to avoid: no information available

Hazardous decomposition products: no information available
Polymeryzation: hazardous polymerization does not occur

Section 10- Toxicological information

Acute toxicity

Principle route of exposure/potential health effects

Eyes: slightly irritating to the eyes
Skin: no known significant effects or critical hazards
Inhalation: no known significant effects or critical hazards
Ingestion: no known significant effects or critical hazards

Specific effects

Carcinogenic effects: no information available
Mutagenic effects: no information available
Reproductive toxicity: no information available
Sensitization: no information available

Section 11- Ecological information

Environmental precautions: no known significant effects or critical hazards
Products of degradation these products are carbon oxides

Section 12- Disposal considerations

Waste disposal: the generation of waste should be avoided or minimized wherever possible. Avoid dispersal of spilled material, runoff and contact with soil, waterways, drains and sewers. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional and local authority requirements.

Section 13- Other information

The above information was acquired by diligent search and/or investigation and the recommendations are based on prudent application of professional judgment. The information shall not be taken as being all inclusive and is to be used only as a guide. All materials and mixtures may present unknown hazards and should be used with caution. Since Dendritics SAS Company cannot control the actual methods, volumes, or conditions of use, the company shall not be held liable for any damages or losses resulting from the handling or from contact of the product as described herein.

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Material Safety Data Sheet for Tris-NaCl
(Uncoupled antibodies)

Dendritics Material Safety Data Sheets (MSDS) comply with OSHA guidelines containing information regarding hazardous chemicals in our products. Many of our products are mixtures of chemicals in which the hazardous ingredient is less than the exposure limit set by OSHA.

Product identification

Chemical name: Sodium chloride CAS No: 7647-14-5 and Tris CAS No: 77-86-1

Physical data

Appearance: clear, colorless
Physical state: liquid
Odor: no information available

Stability

Stable under recommended storage conditions
Incompatible with strong oxidizing agents

Toxicology

Irritating to eyes, skin, and respiratory system.
Causes irritation by ingestion.

Personal protection

Avoid contact with skin, eyes and clothing.
Use personal protective equipment.
Ensure adequate ventilation.

First aid measures

Inhalation: move to fresh air. If breathing becomes difficult, give oxygen.
Ingestion: clean mouth with water and afterwards drink plenty for water.
Skin contact: wash skin with soap and water.
Eye contact: flush eyes with plenty of water for at least 15 minutes and consult a physician.

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