



**Intended use: Immortalization of human B lymphocytes from peripheral blood samples**

**Background:**

Several approaches have been developed by scientists aiming to study the physiology of circulating B cells. They are generally limited both by the low number of B cells (8-10% of peripheral blood mononuclear cells) and by their low capacity to enter cell cycle. The most successful approaches of these last decades were the CD40 system (1), a culture technique allowing long-term growth of B lymphocytes, and B cell transformation using the Epstein-Barr virus (EBV) (2;3;4). However, the efficiency of these methods remains limited, targeting only 1% (CD40) and 1/10<sup>6</sup> (EBV) of the B lymphocytes. An improved method has recently been reported but targeting only a very restricted IgG<sup>+</sup>CD22<sup>+</sup> B cell population (5).

Dendritics has developed the Kit HuBBB<sup>®</sup> to improve the above approaches. The Kit HuBBB allows the combination of a strong B cell activation and an efficient B cell transformation using EBV viral suspension prepared according to proprietary procedures. The Kit HuBBB is intended to target the largest B cell population from peripheral blood, to obtain immortalized B cells representative of the overall peripheral B repertoire. Under the best conditions, the kitHuBBB<sup>®</sup> allows the immortalization of up to 20% of the starting B cell population.

**Kit sizes**

The kit HuBBB<sup>®</sup> is designed to be easy to handle, and packaged in 3 different sizes to be affordable for all the scientists

Reference	Starting PBMC	Estimated B independent clones	96w plates
DDXK-HuBBB-10	10 <sup>7</sup>	5.10 <sup>4</sup> -2.10 <sup>5</sup>	10
DDXK-HuBBB-5	5.10 <sup>6</sup>	2,5.10 <sup>4</sup> -10 <sup>5</sup>	5
DDXK-HuBBB-2	2.10 <sup>6</sup>	10 <sup>4</sup> -0,5.10 <sup>5</sup>	2

**Kit contents:**

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

color	vial n°	designation	DDXK-HUBBB-10	DDXK-HUBBB-5	DDXK-HUBBB-2	storage
green	1	support 1	freeze-dried	freeze-dried	freeze-dried	-20°C
	2	mAb1	200µl	100µl	50µl	-20°C
	3	mAb2	200µl	100µl	50µl	-20°C
orange	4	EBV1	2ml	1ml	0,5ml	-80°C
yellow	5	support2	freeze-dried	freeze-dried	freeze-dried	-20°C
	6	mAb3	200µl	100µl	50µl	-20°C
	7	EBV2	2ml	1ml	0,5ml	-80°C
<b>booster reagent</b>			67ml	35ml	15ml	-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

**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<sup>+</sup> monocytes.
- \*The efficiency of the process can be significantly affected for blood samples containing less than 2% of CD19<sup>+</sup> B lymphocytes.
- \*The efficiency of the process can be significantly affected by EBV suspension freeze / thaw cycles
- \*Depending on the type of incubator, if a significant reduction of the medium volume is observed (evaporation) between Day3-Day8 and Day 10-18, the final volume can be adjusted by addition of fresh medium at any time.

**Experimental procedure**

The different vials are regrouped by color according to the day of use:

**Day-1: green vials**

**Day 0: orange vial**

**Day 9: yellow vials**

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

**Day-1: green vials****Complete medium preparation**

<b>Complete medium</b>	<b>DDXK-HUBBB-10</b>	<b>DDXK-HUBBB-5</b>	<b>DDXK-HUBBB-2</b>
DMEMF12	500ml	250ml	100ml
L-Glutamine*	20ml	10ml	4ml
Booster reagent	67ml	35ml	15ml

\*8mM final concentration.

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

Reconstitute vial1 with 2ml of complete medium

**MIX A preparation**

<b>MIX A</b>	<b>DDXK-HUBBB-10</b>	<b>DDXK-HUBBB-5</b>	<b>DDXK-HUBBB-2</b>
Complete medium	100ml	50ml	20ml
Reconstituted vial 1	2ml	2ml	2ml
Vial 2	200µl	100µl	50µl
Vial 3	200µl	100µl	50µl

Distribute 100µl/well of **MIX A** in 96 wells culture plates.

Incubate overnight at 37°C, under 5 %CO<sub>2</sub>

**Day 0: orange vial****PBMC isolation**

PBMC isolation by density (d=1.077) gradient centrifugation (Ficoll-Hypaque, Pancoll, MSL or equivalent).

Briefly:

- Obtain sterile blood in ACD tubes (yellow top)
- Dilute 1:1 with PBS in (50ml) tubes
- Underlay 25ml of diluted blood with 15 ml of Ficoll
- Spin at 1600 rpm for 30 min, brake off, RT
- Carefully transfer the PBMC ring to a new (50ml) tube
- Add PBS up to 50ml to the PBMC
- Spin at 1600 rpm for 10 min, RT
- Discard supernatant and resuspend pellet in 50ml of PBS
- Spin at 1100 rpm for 20 min, RT

Adjust cell concentration at **10<sup>5</sup> PBMC/ml** in complete medium.

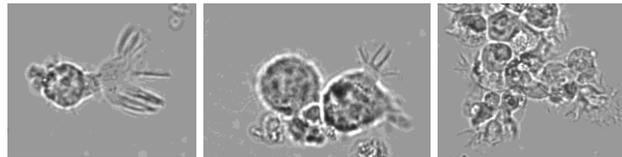
## MIX B preparation

<b>MIX B</b>	<b>DDXK-HUBBB-10</b>	<b>DDXK-HUBBB-5</b>	<b>DDXK-HUBBB-2</b>
PBMC suspension	100ml	50ml	20ml
Vial 4	2ml	1ml	0,5ml

Take out the culture plates from the incubator and distribute 100µl/well of **MIX B**

Incubate overnight at 37°C, under 5 %CO<sub>2</sub>

### Days 3 to 8: microscopic observation



Microscopic observation during the time course of the culture

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

### Day 9: yellow vials

Reconstitute vial5 with 2ml of complete medium

## MIX C preparation

<b>MIX C</b>	<b>DDXK-HUBBB-10</b>	<b>DDXK-HUBBB-5</b>	<b>DDXK-HUBBB-2</b>
Complete medium	100ml	50ml	20ml
Reconstituted vial 5	2ml	2ml	2ml
Vial6	200µl	100µl	50µl
Vial 7	2ml	1ml	0,5ml

Take out the culture plates from the incubator

Gently (avoid aspirating cells) discard 100µl of culture supernatant from each well.

Add 100µl of **MIX C** /well

### Day 20: Culture expansion

Transfer the best proliferating wells into 24 wells plates in complete medium

Expand the cultures with medium change 2-3 times a week.

When a sufficient number of cells is reached, cells can be either directly frozen for storage or diluted for cloning before storage.

For application studies, cells are thawed according to standard procedures, cultured and amplified in complete medium to be available for further studies studies.

**Of note:** an average of 10<sup>6</sup> cells should be obtained from a well (24 wells plates) within 2 weeks.

### **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, Gergely P, Jondal M, Klein G, Britton S. *Nature*. 1977 May 5;267(5606):52-4.
- 3-EB virus-induced B lymphocyte cell lines producing specific antibody. Steinitz M, Klein G, Koskimies S, Makel O. *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, Vrolix K, Martinez-Martinez P, Losen M, Meulemans E, De Baets MH, Stinissen P, Somers V. *J Autoimmun*. 2010 Sep;35(2):130-4

## Material Safety Data Sheet (EBV suspension)

The Epstein-Barr virus provided in the DDXK-HuBBB kit is of the B95-8 strain, produced as a supernatant of a subclone (established by Dendritics company) of the original 883L human cell line (licensed by Schering-Plough). This viral suspension is intended to immortalize primate B lymphocytes and required to be handled as a potentially biohazardous material under biosafety level 2 containment. The cell culture supernatant provided in this kit contains an attenuated form of the Epstein-Barr virus.

The safety data listed below are related to EBV positive biological samples resulting from a natural EBV infection and are given for information.

### Section 1-Infectious agent

**Name:** Epstein-Barr virus

**Synonym or cross reference:** EBV, infectious mononucleosis (IM), glandular fever, Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), human herpes virus (HHV) 4.

**Characteristics:** double-stranded linear DNA, 120-150nm diameter, enveloped, icosahedral; types A and B; Herpesviridae, subfamily of gammaherpesvirinae.

### Section 2- Health hazard

**Pathogenicity:** Infectious mononucleosis-acute viral syndrome with fever, sore throat, splenomegaly and lymphadenopathy; one to several weeks, rarely fatal/ Burkitt's lymphoma- monoclonal tumor of B cells; AIDS patients (25-30% are EBV related)/Nasopharyngeal carcinoma-malignant tumor of epithelial cells of the nasopharynx involving adults between 20 and 40 years, reactivation causes lymphoproliferative disorders (LPD) and posttransplant lymphoproliferative disorders (PTLDs).

**Epidemiology:** EBV infects 80-90% of all adults worldwide; mononucleosis is common in early childhood worldwide, typical disease occurring in developed countries mainly in young adults; Burkitt's lymphoma is worldwide but hyperendemic in highly malarial areas such as tropical Africa; carcinoma is worldwide but highest in Southeast Asia and China, especially in males; EBV plays an important pathogenic role in approximately 97% of BL cases in Africa and Papua New Guinea.

**Host range:** Humans

**Infectious dose:** not known

**Mode of transmission:** Mononucleosis-person-to-person by oropharyngeal route via saliva, possible spread via blood transfusion (not important route); Burkitt's lymphoma-primary infection occurs early in life or involves immunosuppression and reactivation of EBV later, malaria an important co-factor; NPC infection occurs in early life and reactivation later with epithelial invasion.

**Incubation period:** IM-4 to 6 weeks; BL-2 to 12 years from primary infection, shorter in AIDS patients; NPC-unknown. Communicability: IM-prolonged period, up to 1 year or more, 15-20% of EBV antibody-positive adults are oropharyngeal carriers; tumors are not communicable.

### Section 3- Dissemination

**Reservoir:** humans

**Zoonosis:** none

**Vectors:** none

#### Section 4-Viability

**Drug susceptibility:** Nucleoside analogs (Acyclovir, Ganciclovir, and Famciclovir) inhibit the replication of EBV.

**Susceptibility to disinfectants:** susceptible to disinfectants-1% sodium hypochlorite, 70% ethanol, glutaraldehyde, formaldehyde.

**Physical inactivation:** inactivated by heat (50-60°C for at least 30 min).

**Survival outside host:** survives at room temperature for a few days, 2-3 days at refrigeration temperature, -70°C many years.

#### Section 5-Medical

**Surveillance:** monitor for symptoms; confirm serologically (heterophilic antibody titre, EBV VCA IgM and IgG titer by Elisa) positive in 85-90% of IN patients.

**First aid/treatment:** no specific treatment; steroids may be of some value in severe cases.

**Immunization:** subunit vaccines in trial stage

**Prophylaxis:** none

#### Section 6- Laboratory hazards

**Laboratory-acquired infections:** very rare.

**Source/specimens:** clinical specimens-nasopharyngeal secretions, blood.

**Primary hazards:** Ingestion, accidental parenteral inoculation, droplet exposure of the mucous membranes, inhalation of concentrated aerosolized materials.

**Special hazards:** none.

#### Section 7- Recommended precautions

**Containment requirements:** biosafety level 2 practices, containment equipment and facilities for activities utilizing known of potentially infectious clinical materials or cultures.

**Protective clothing:** laboratory coat; gloves when direct contact with infectious materials is unavoidable.

**Other precautions:** procedures that are likely to generate aerosols should be conducted in a biosafety cabinet.

#### Section 8- Handling information

**Spills:** allow aerosols to settle; wear protective clothing; gently cover spill with paper towel and apply 1% sodium hypochlorite, starting at perimeter and working towards the center; allow sufficient contact time (30 min) before cleanup.

**Disposal:** decontaminate before disposal; steam sterilization, chemical disinfection, incineration

**Storage:** in sealed containers that are appropriately labeled.

#### Section 9- Additional information

The above information is believed to be accurate but does not purport to be all inclusive and shall be used only as a guide. Dendritics shall not assume any liability whatsoever for the accuracy or completeness of the information contained herein. Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

**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](mailto: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 for firefighters:** wear self-contained breathing apparatus and protective suit

**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 contain 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  
**Polymerization:** 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.

**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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## **WORKFLOW**

### **DAY -1**

- Distribute 100µL/well of **MIX A**
- Incubate culture plates at 37 C, 5%CO<sub>2</sub>

### **DAY 0**

- Isolate PBMC from sterile blood collected in ACD tubes (yellow top)
- Take out the plates and add 100µl/well of **MIX B**
- Incubate culture plates at 37 C, 5%CO<sub>2</sub>

### **DAYS 3 to 8**

- Proliferating patches of hairy cells should be observed under microscope  
*Total medium volume can be adjusted by adding fresh medium if necessary*

### **DAY 9**

- Take out (without shaking) the culture plates from the incubator
- Carefully discard 100µl of the culture supernatant
- Add 100µl/well of **MIX C**

### **DAY 20**

- Transfer the best proliferating wells il 24wells plates in complete medium
- Change medium 2-3 times /week