**Human Blood B Booster® Kit**

(DDXK-HuBBB)

**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 1/10^6 (EBV) of the B lymphocytes. An improved method has recently been reported but targeting only a very restricted IgG^+^CD22^+^ B cell population (5).

Dendritics has developed the Kit HuBBB® 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® allows the immortalization of up to 20% of the starting B cell population.

**Kit sizes**
The kit HuBBB® is designed to be easy to handle, and packaged in 3 different sizes to be affordable for all the scientists.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Starting PBMC</th>
<th>Estimated B independent clones</th>
<th>96w plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>DDXK-HuBBB-10</td>
<td>10⁷</td>
<td>5.10⁴-2.10⁵</td>
<td>10</td>
</tr>
<tr>
<td>DDXK-HuBBB-5</td>
<td>5.10⁶</td>
<td>2,5.10⁴-10⁵</td>
<td>5</td>
</tr>
<tr>
<td>DDXK-HuBBB-2</td>
<td>2.10⁶</td>
<td>10⁴-0.5.10⁵</td>
<td>2</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>color</th>
<th>vial n°</th>
<th>designation</th>
<th>DDXK-HuBBB-10</th>
<th>DDXK-HuBBB-5</th>
<th>DDXK-HuBBB-2</th>
<th>storage</th>
</tr>
</thead>
<tbody>
<tr>
<td>green</td>
<td>1</td>
<td>support1</td>
<td>freeze-dried</td>
<td>freeze-dried</td>
<td>freeze-dried</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>mAb1</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>mAb2</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
<td>-20°C</td>
</tr>
<tr>
<td>orange</td>
<td>4</td>
<td>EBV1</td>
<td>2ml</td>
<td>1ml</td>
<td>0,5ml</td>
<td>-80°C</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>support2</td>
<td>freeze-dried</td>
<td>freeze-dried</td>
<td>freeze-dried</td>
<td>-20°C</td>
</tr>
<tr>
<td>yellow</td>
<td>6</td>
<td>mAb3</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
<td>-20°C</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>EBV2</td>
<td>2ml</td>
<td>1ml</td>
<td>0,5ml</td>
<td>-80°C</td>
</tr>
</tbody>
</table>

**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)

**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 by blood samples containing less than 2% of CD19^+^ 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.

### Complete medium preparation

<table>
<thead>
<tr>
<th>Complete medium</th>
<th>DDXK-HUBBB-10</th>
<th>DDXK-HUBBB-5</th>
<th>DDXK-HUBBB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMEMF12</td>
<td>500ml</td>
<td>250ml</td>
<td>100ml</td>
</tr>
<tr>
<td>L-Glutamine*</td>
<td>20ml</td>
<td>10ml</td>
<td>4ml</td>
</tr>
<tr>
<td>Booster reagent</td>
<td>67ml</td>
<td>35ml</td>
<td>15ml</td>
</tr>
</tbody>
</table>

*8mM final concentration.

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

Reconstitute vial 1 with 2ml of complete medium

### MIX A preparation

<table>
<thead>
<tr>
<th>MIX A</th>
<th>DDXK-HUBBB-10</th>
<th>DDXK-HUBBB-5</th>
<th>DDXK-HUBBB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete medium</td>
<td>100ml</td>
<td>50ml</td>
<td>20ml</td>
</tr>
<tr>
<td>Reconstituted vial 1</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
</tr>
<tr>
<td>Vial 2</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
</tr>
<tr>
<td>Vial 3</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
</tr>
</tbody>
</table>

Distribute 100µl/well of MIX A in 96 wells culture plates.
Incubate overnight at 37°C, under 5%CO₂

### 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 $10^5$ PBMC/ml in complete medium.
**MIX B preparation**

<table>
<thead>
<tr>
<th>MIX B</th>
<th>DDXK-HUBBB-10</th>
<th>DDXK-HUBBB-5</th>
<th>DDXK-HUBBB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMC suspension</td>
<td>100ml</td>
<td>50ml</td>
<td>20ml</td>
</tr>
<tr>
<td>Vial 4</td>
<td>2ml</td>
<td>1ml</td>
<td>0,5ml</td>
</tr>
</tbody>
</table>

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

Incubate overnight at 37°C, under 5% CO₂.

**Days 3 to 8:** microscopic observation

![Microscopic observation during the time course of the culture](image)

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

**Day 9:** yellow vials

Reconstitute vial 5 with 2ml of complete medium

**MIX C preparation**

<table>
<thead>
<tr>
<th>MIX C</th>
<th>DDXK-HUBBB-10</th>
<th>DDXK-HUBBB-5</th>
<th>DDXK-HUBBB-2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete medium</td>
<td>100ml</td>
<td>50ml</td>
<td>20ml</td>
</tr>
<tr>
<td>Reconstituted vial 5</td>
<td>2ml</td>
<td>2ml</td>
<td>2ml</td>
</tr>
<tr>
<td>Vial 6</td>
<td>200µl</td>
<td>100µl</td>
<td>50µl</td>
</tr>
<tr>
<td>Vial 7</td>
<td>2ml</td>
<td>1ml</td>
<td>0,5ml</td>
</tr>
</tbody>
</table>

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.

**Of note:** an average of 10⁶ cells should be obtained from a well (24 wells plates) within 2 weeks.

**References:**

Material Safety Data Sheet (EBV suspension)

The Epstein-Barr virus provided in the DDKX-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 handle 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 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
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 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%CO₂

**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%CO₂

**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