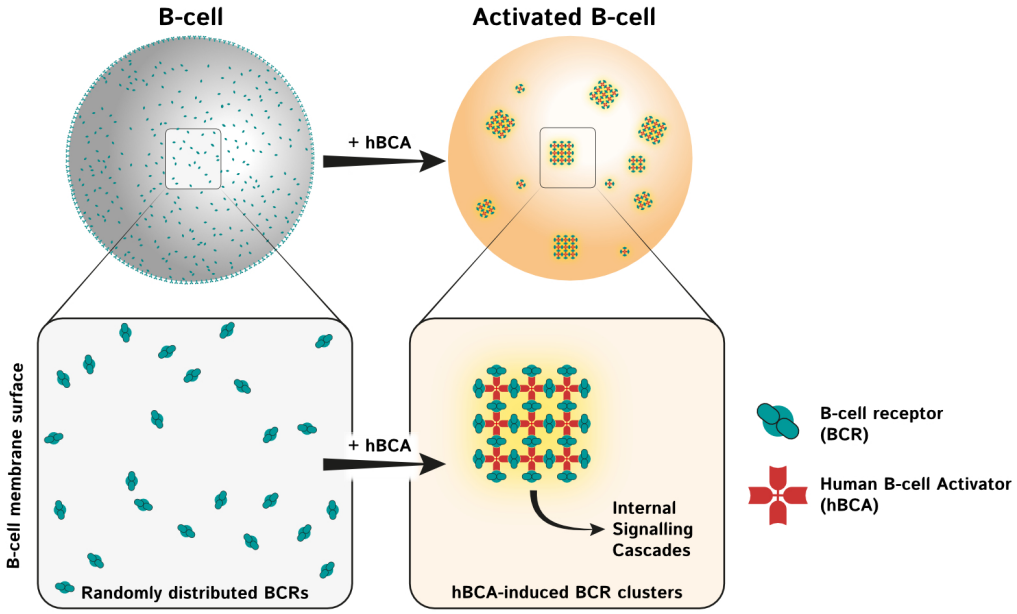
Stimulation of human B-cells using Human B-cell Activator

A. Introduction

NanoTag's **Human B-cell Activator (hBCA)** is a tetramerized **single-domain antibody (sdAb)** specifically designed to crosslink surface-bound **human IgM (BCR µ-chain)** and promote efficient nanoscale receptor clustering. This clustering drives rapid proximal signaling cascades, including phosphorylation events, calcium flux, and MAPK/NF-κB activation.



By leveraging a tetrameric sdAb format, the reagent provides robust BCR clustering without the need for secondary antibodies and consistently yields a **faster and stronger activation response** compared with conventional IgM crosslinking antibodies. The Fc-free design avoids Fc receptor–mediated artifacts. Recombinant production guarantees identical performance with minimal batch-to-batch variations.

Product Format

Product Name	Cat. No.	Amount	Format
Human B-cell Activator (hBCA)	N1170	250 µg	Recombinant protein, Lyophilized from PBS.

B. Compatibility and Applications

- **Cell Types:**
  - Primary human B-cells (e.g., from PBMCs)
  - IgM^+ B-cell lines
  - *Note:* Does not cross-react with mouse or rat IgM molecules.

- **Applications:**
  - Signaling pathway studies
  - Activation marker analysis
  - BCR clustering imaging
  - Proliferation assays
  - Cytokine readouts
  - Functional co-culture models

C. Example Reference Protocol

The following protocol outline is based on published and commonly used antibody-based activation methods. It is provided for reference and general conceptualization of the experimental workflow only. It is required to optimize the workflow for each experimental setup.

I. Cell preparation

Sources:

- B-cells from human PBMCs or
- IgM^+ B-cell lines (e.g., Raji)

Culture conditions:

- RPMI-1640 medium supplemented with 10% FBS (or equivalent), 2 mM L-glutamine, 100 U/mL penicillin, and 100 µg/mL streptomycin
- Incubation at 37 °C with 5% CO_2.

Isolation:

- If required, naïve B-cells can be obtained using negative-selection kits.

II. Stimulation

Human B-cell Activator (hBCA) will also recognize soluble human IgM. Serum proteins must be removed by washing before stimulation.

Reagent:

- Human B-cell Activator (hBCA)

Principle:

- Depending on the desired activation endpoint, the reagent is added directly to the culture medium to crosslink surface IgM and cluster BCRs.

Reported ranges:

- Concentration range 0.5-10 µg/mL
- Acute signaling can be observed within 15 minutes.
- Long-term proliferation and differentiation may require 1–4 days of stimulation.
- Optimal concentration and timing should be determined empirically.

III. Monitoring Cell Activation

• **Flow cytometry:**	Measure activation markers such as CD69 and CD86; Monitor kinetics over time as required.
• **Proliferation assays:**	Assess DNA synthesis incorporation using published methods.
• **Cytokine assays:**	Detect cytokine secretion (e.g., IL-2) in culture supernatants.
• **Cell viability:**	Determine ration of viable cells using live/dead dyes.
• **BCR internalization:**	Visualize receptor dynamics by scopy or flow cytometry.

IV. Suggested Controls

- **Negative control:** Untreated cells or cells treated with monovalent sdAb anti-Human IgM (#N1101)
- **Comparison products:** Conventional IgM crosslinkers, e.g., Goat anti-human IgM $F(ab')_2$

V. Specificity

Species:	Specific for human IgM; does not bind mouse or rat IgM. Non-human primates have not been tested.
Isotype:	Designed specifically for IgM^+ B-cells; Cells dominated by other BCR isotypes may show different responses.

Human B-cell Activator is intended exclusively for research applications, not for diagnostic or therapeutic use!

For further information concerning this protocol please contact us at
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