BI
Biological Industries

TECHNICAL INFORMATION

BIOTARGET-1 SERUM-FREE MEDIUM

Cat. No. 05-080-1

Introduction

The BIOTARGET-1 formulation has been developed specifically for use with mononuclear cells (lymphocytes and monocytes) from peripheral blood. In work with these cells and their sub-populations, it is critical to optimize and define the media formulation as well as pH and temperature.

In most cases at present, these cells are grown in convential media, supplement with human serum (A, AB) or foetal bovine serum. However, the use of serum suffers from the following disadvantages:

1. The serum may contain non-specific growth factors, which interfere with complete activation in the desired direction.
2. The serum may contain inhibitors which will limit activation of the lymphocytes.
3. Lot to lot variation is certain.
4. Pathogens may be introduced via the serum.
5. The evaluation of the antigenic reaction, such as the quantity of the lymphokines generated, and the reaction of the lymphokines to hormones and the growth factors are all more accurate in the absence of serum.

We have, therefore, developed BIOTARGET-1 as a serum-free alternative for these purposes. In the course of this work, hundreds of possible formulation variations have been screened, while comparing directly results obtained with human serum or foetal bovine serum – supplemented conventional medium, as well as with a competitor's commercial serum-free formulation. Initial screening was carried out on the basis of cell viability and reaction to induction by mitogens. In a later stage several more specific characteristics were evaluated before selecting a final formulation.

Evaluation Protocols

Several examples of evaluation protocols by which BIOTARGET-1 was selected follow.

The preparation of mononuclear cells was performed as follows. Pooled peripheral blood was received from Israel's Central Blood Bank, after stringent testing for Hepatitis B and HIV viruses. The cells were centrifuged in tubes containing Ficoll-paque. The mononuclear cells were concentrated in the layer between the Ficoll and the plasma. The cells were removed with a pipette and washed three times in PBS with centrifugation. The cells were divided into microwells containing the medium tested, at a concentration of $2x10^5$ cells per well.

1. **Mitogenic Activation of Mononuclear Cells (Figures 1-9)**
   Activation was evaluated with different mitogens such as PHA, CON.A and
   OKT-3. Proliferation was checked by measurements of the uptake of radioactive thymidine. The mitogens were added in varying concentrations and thymidine uptake was determined over several days, in order to fully evaluate the specific medium formulation.

2. **Activation of Mononuclear Cells with Lymphoid Cells (Figures 10-11)**
   The activation of the mononuclear cells was carried out using lymphoid cells of various kinds, such as: JURKAT, RAJI, MOLT-4, and BA. Varying ratios between the tumor cells and the mononuclear cells were examined, and the proliferation was measured using radioactive thymidine.

3. **Production of Lymphokines by Activated Mononuclear Cells (Figures 12-15)**
   The levels of the lymphokines IL-2 and IL-3 were measured in the culture of the mononuclear cells after activation with various mitogens. IL-2 production was measured with the help of the CTLL-2 cell line. These are cytotoxic T-cells from mice, which grow only in the presence of IL-2 in the culture medium.

4. **Cytotoxicity (Tables 1 and 2)**
   Mononuclear cells were seeded at a concentration of 10^6 cells per well together with RAJI cells which had been treated with mitomycin C. Varying ratios of the two cell types were examined. At the conclusion of the activation (5-7 days), the lymphocytes were collected, centrifuged, suspended in medium and seeded in microwells in order to measure proliferation and cytotoxicity. RAJI cells were labeled with radioactive chromium (100 ci in a volume of 0.2 ml), washed three times, suspended at a concentration of 10^5 cells per ml, and divided into microwells containing the above activated lymphocytes. After 18 hours incubation, the cytolytic activity was evaluated by measuring the radioactive chromium released from the target (RAJI) cells.

Applications for BIOTARGET-1

The applications for the use of this serum-free formulation are numerous and include:

1. Activation of mononuclear cells with the aid of various mitogens (PHA, CON.A, OKT-3)
2. Activation of mononuclear cells with lymphoid cells (RAJI, PEER, BA, MOLT-4, JURKAT)
3. Production of IL-2 and IL-3 from mononuclear cells
4. Long-term culture of mononuclear cells after activation
5. Activation of mononuclear cells with interleukin-2 in order to generate LAK or TIL cells
6. Activation of mononuclear cells in order to generate natural killer cells (NK)
7. Activation of mononuclear cells in order to generate cytotoxic T cells
8. Activation of macrophages
9. Research on the influence of various cytokines on the production of sub-populations of mononuclear cells
10. Proliferation of the HIV virus
11. Proliferation of retroviruses in T cells for the purposes of vaccine development

Figures

1|2 Mitogenic activation of mononuclear cells with CON.A at different concentrations

Figure 1: Day 5

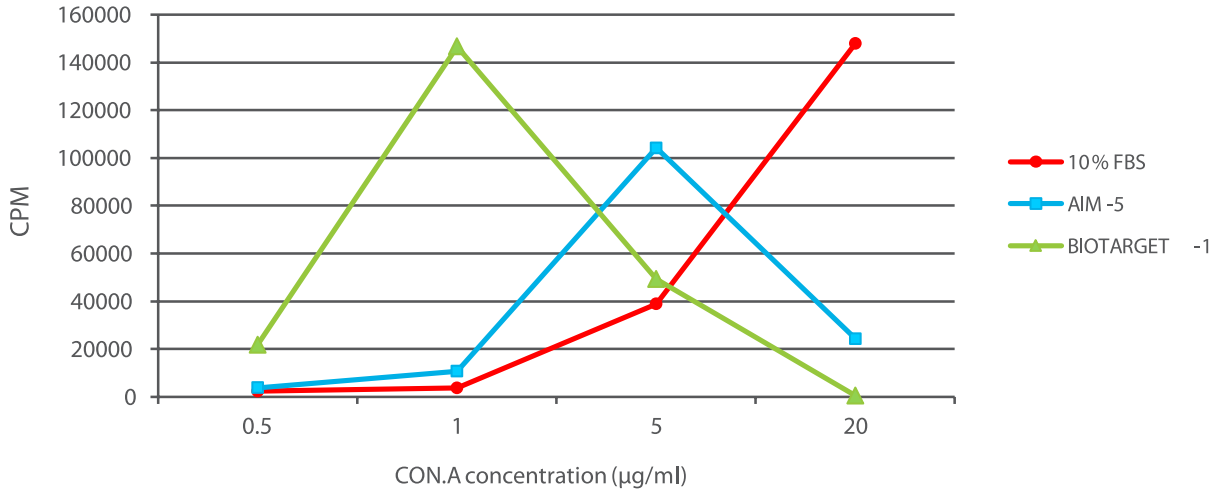
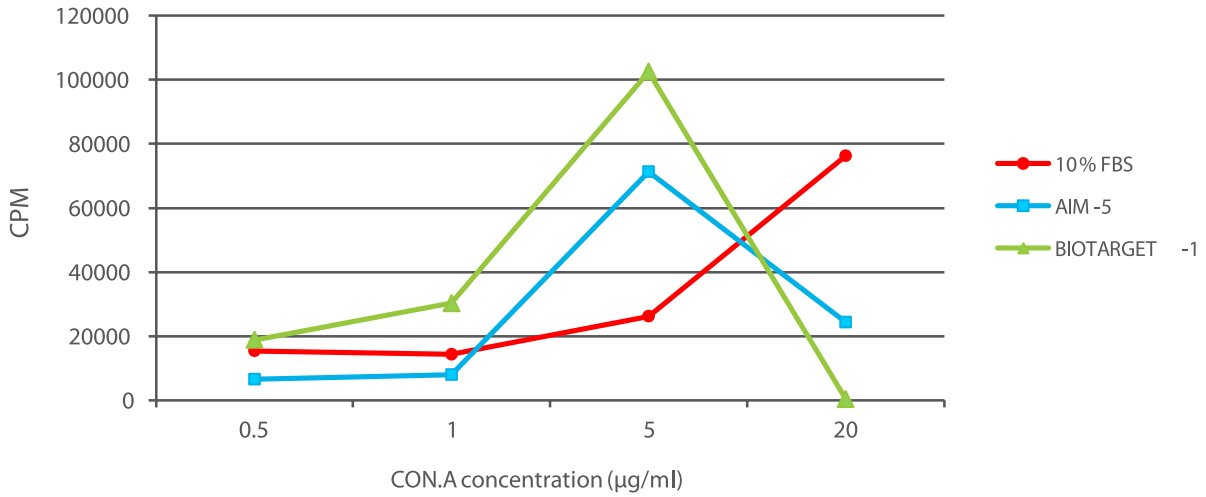


Figure 2: Day 7



Figures

3|4 The Influence of CON.A concentration on the level of activation of mononuclear cells

Figure 3: Day 5

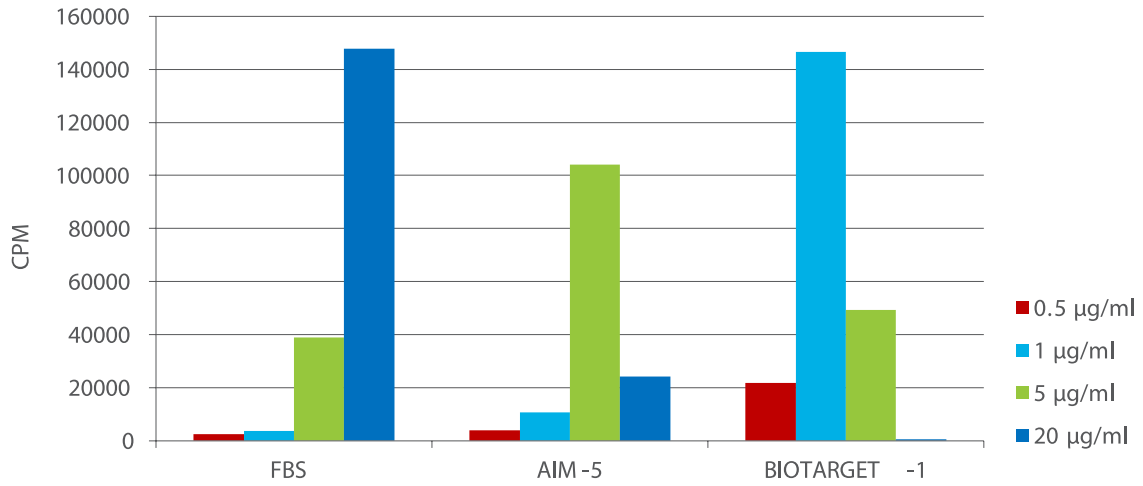
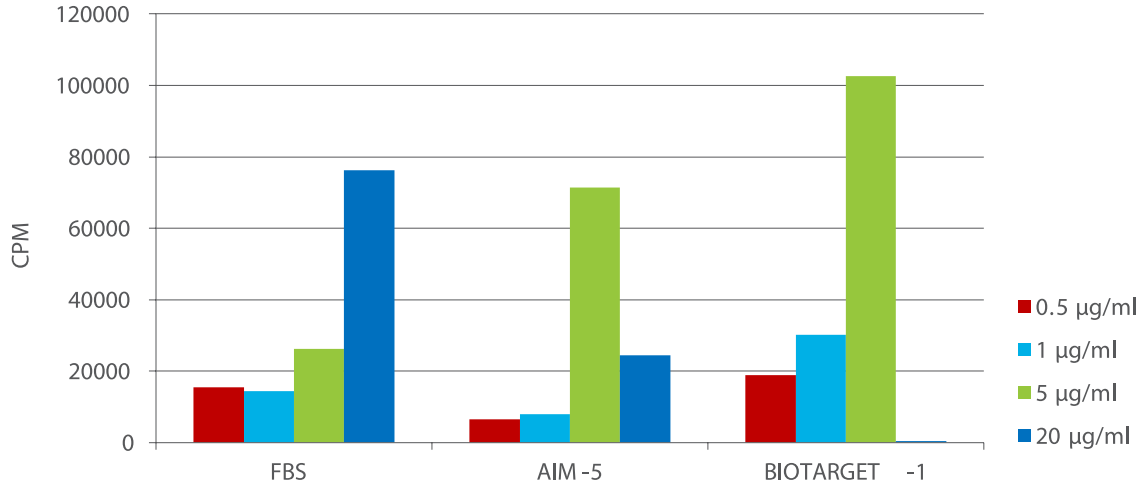


Figure 4: Day 7



Figures

5|6|7|8 Activation of mononuclear cells with PHA at different concentrations

Figure 5: Day 3

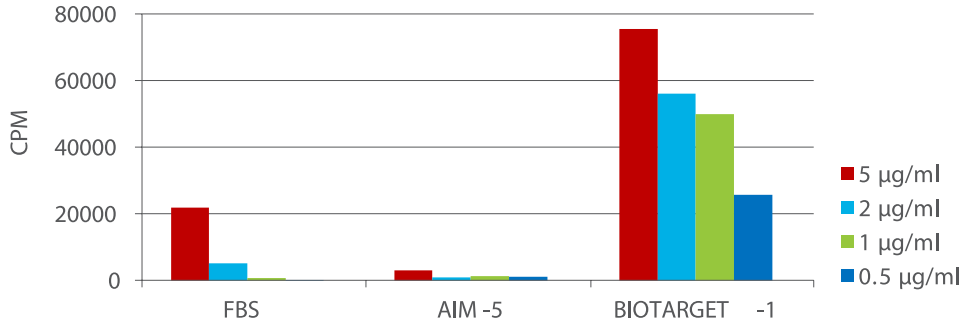


Figure 6: Day 5

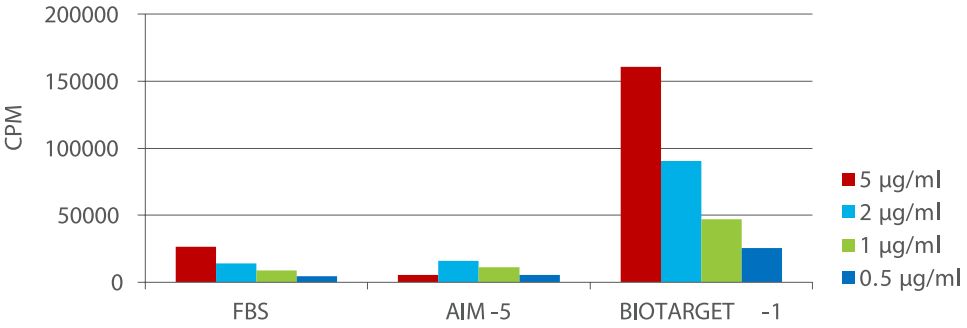


Figure 7: Day 7

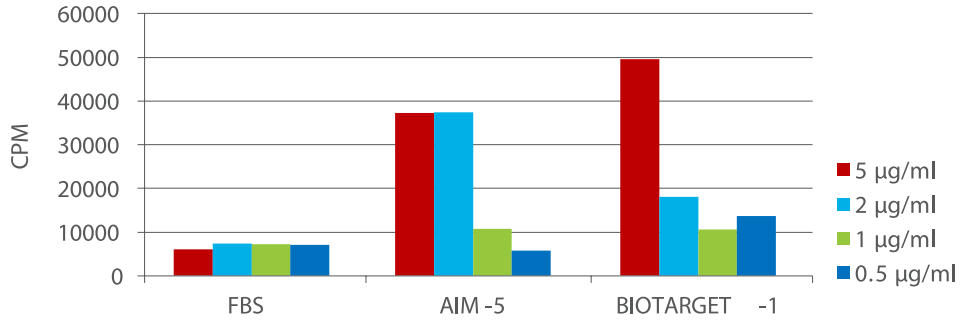
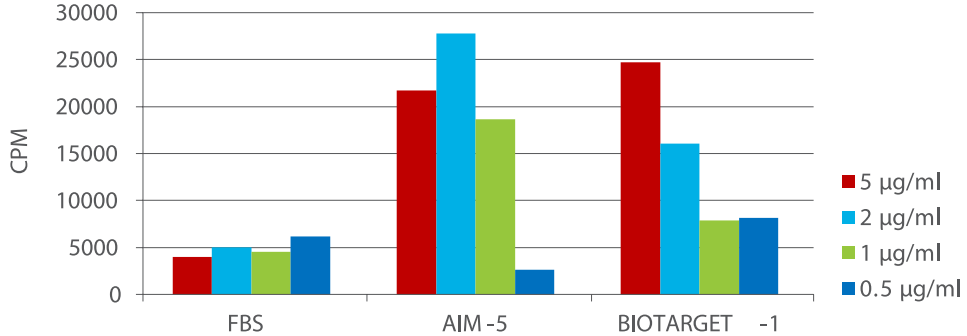


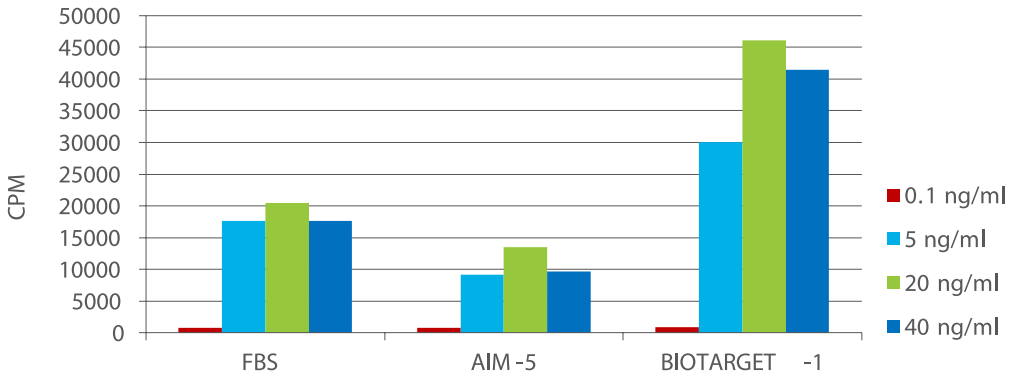
Figure 8: Day 8



Figure

9 Activation of mononuclear cells with OKT-3

Figure 9



Figures

10|11 Activation of mononuclear cells with lymphoid cells

Figure 10: RAJI

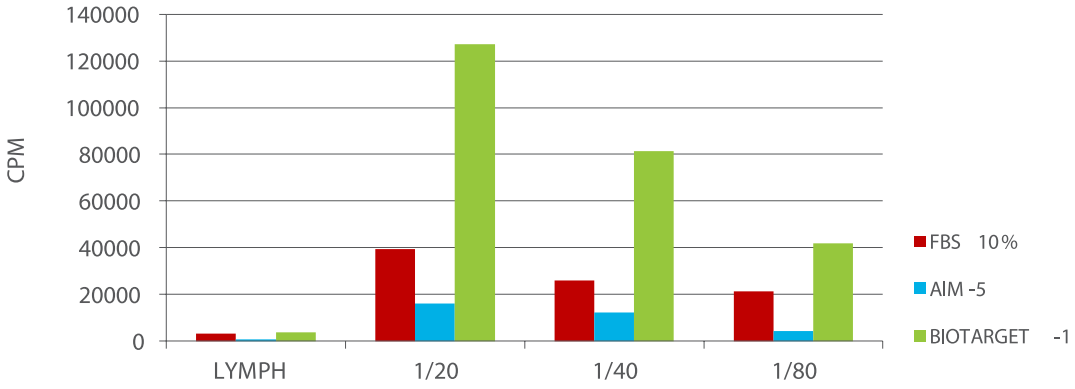
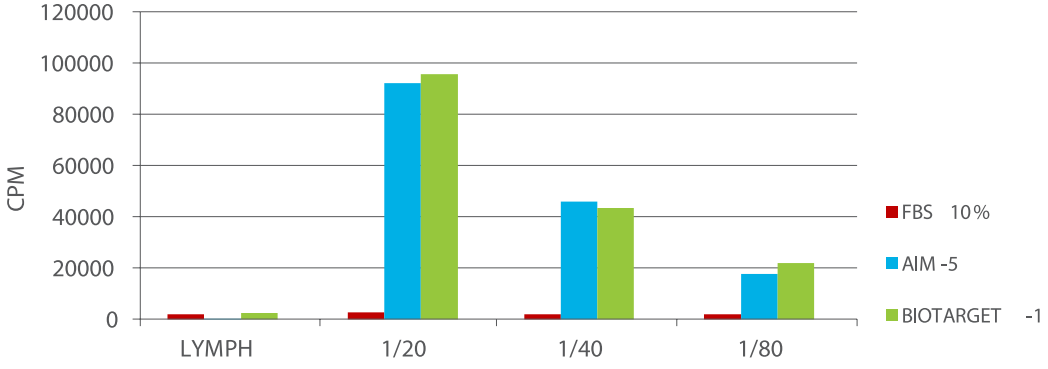


Figure 11: BA



12|13 Production of IL-2 by mononuclear cells that were activated with CON.A or PHA (Supernatant on CTLL cells)

Figure 12: CON.A

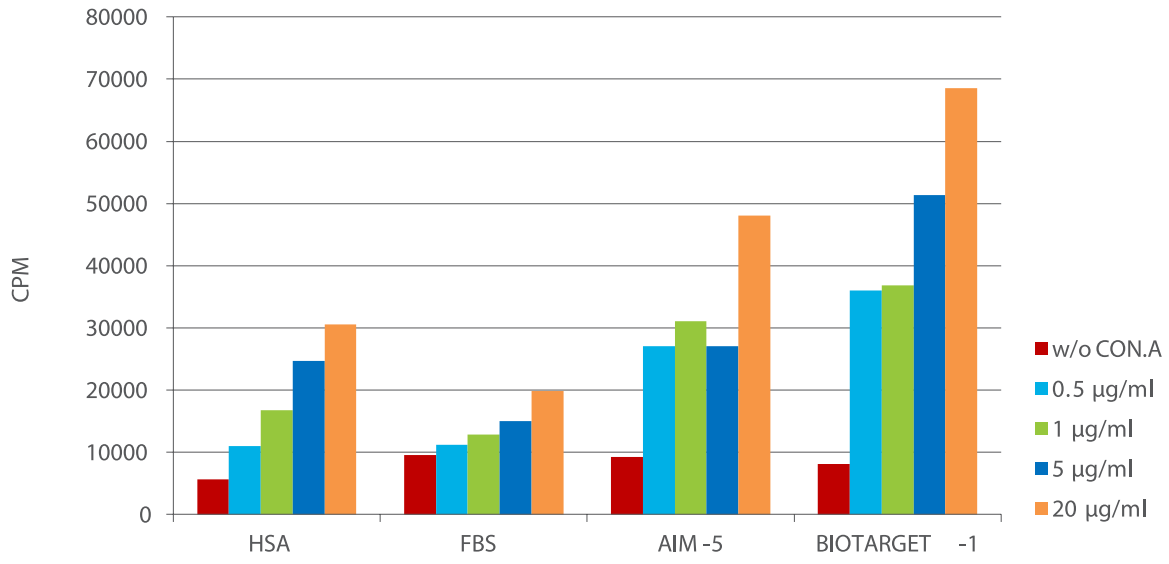
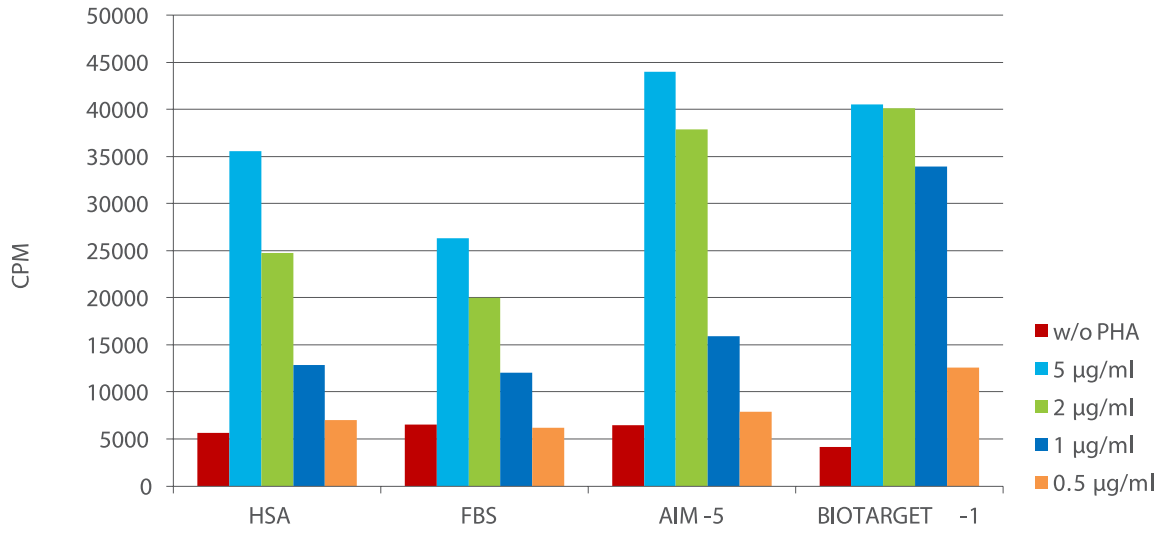


Figure 13: PHA



14|15 Production of IL-3 by mononuclear cells that were activated with CON.A or PHA (supernatant on 32 D-CL-23 cells)

Figure 14: CON.A

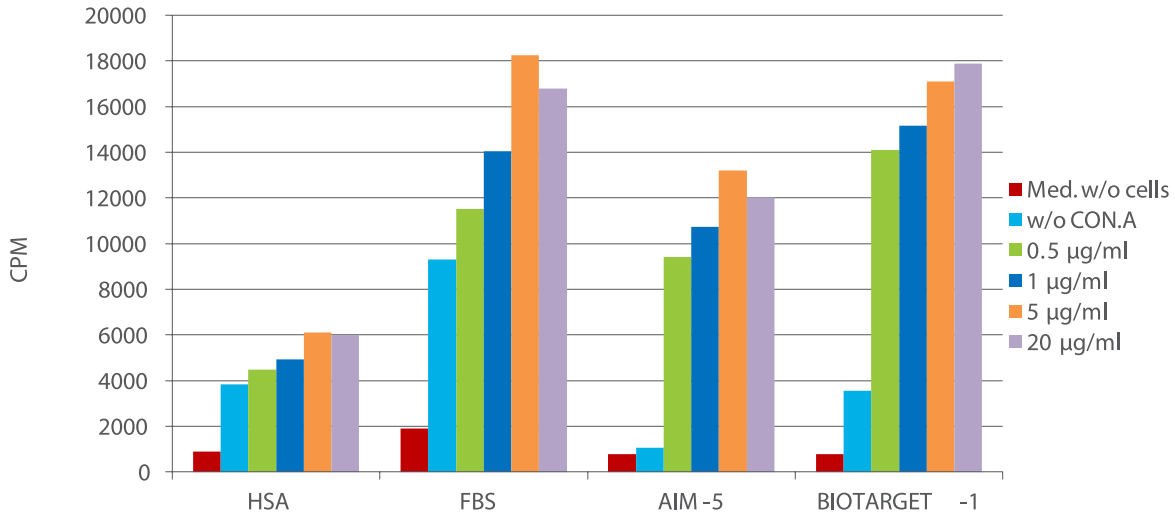
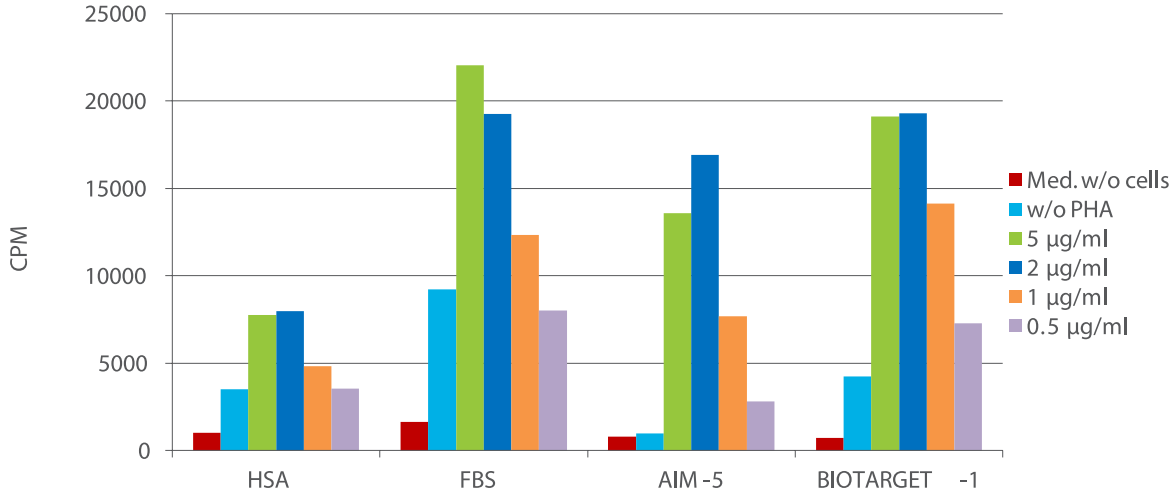


Figure 15: PHA



Tables

1|2 Activation of mononuclear cells with RAJI cells for five days

Table 1

Proliferation of the mononuclear cells.
Results expressed in counts per minute of radioactive Thymidine.

	Mononuclear cells only	Mononuclear cells Ratio RAJI cells	
		1/5	1/10
FBS	3193	1154	4190
AIM-5	361	314	2519
BIOTARGET	2939	771	5680

Table 2

Measurement of Cytotoxicity using RAJI cells as target cells.
Results expressed as percentage of specific release of radioactive Chromium (Total release minus spontaneous release).

	Mononuclear cells Ratio RAJI cells	
	1/5	1/10
FBS	3.0	21
AIM-5	2.5	6.0
BIOTARGET	12	11

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