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APPENDIX

Reagents and buffers

Carbonate buffer (coating buffer)

Na_2CO_3	1.6	g
$NaHCO_3$	2.9	g
NaN_3	0.2	g

Dissolve reagents in 500 ml distilled water and adjust pH to 9.5 with 1N HCl or 1N NaOH, and then fill distilled water up to 1,000 ml. Store at 4°C.

Phosphate buffer saline

NaH_2PO_4 (anhydrous)	0.23	g
Na_2HPO_4 (anhydrous)	1.15	g
NaCl	9.00	g

Dissolve reagents in 500 ml distilled water and adjust pH to 7.2-7.4 with 1N HCl or 1N NaOH, and then fill distilled water up to 1,000 ml. Store at 4°C.

PBS-Tween

Phosphate buffer saline	1,000	ml
Tween 20	500	µl

Mix well and store at 4°C.

5% BSA

Phosphate buffer saline	100	ml
BSA	5	g

Mix well and store at 4°C.

10x TBE buffer

Tris Base	108	g
Boric acid	55	g
0.5 M EDTA pH 8.0	40	ml

Dissolved reagents in 500 ml distilled water and then fill distilled water up to 1,000 ml

Store at room temperature.

6x loading buffer

Formamide	2700	µl
10x TBE	150	µl
Xylene cyanol	0.6	mg
Bromophenol blue	0.6	mg
Distilled water	150	µl

Store at 4°C.

RPMI-10

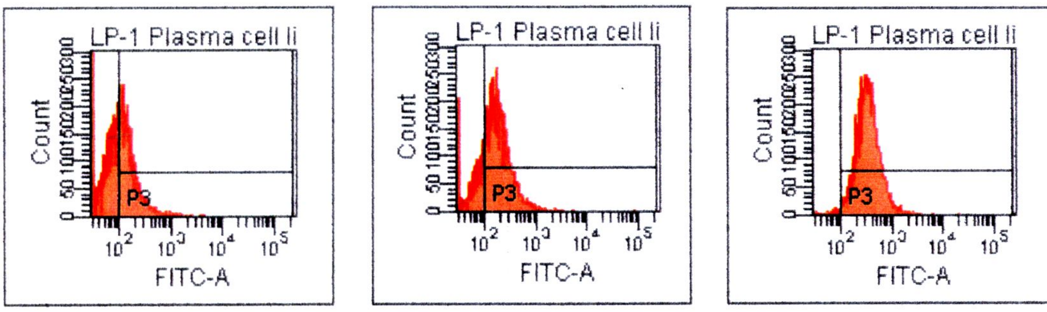
RPMI medium	500	ml
FBS	50	ml
Sodium pyruvate 100X	5	ml
Gentamycin (40 mg/ml)	625	µl

Sterile filtration using 0.45 mM Millipore filter and check sterility by incubation of 2 ml medium in 5% CO_2 at 37°C for a week. Medium was stored at 4°C.

Titration of FITC-conjugated Antibody

Antibody 1:100

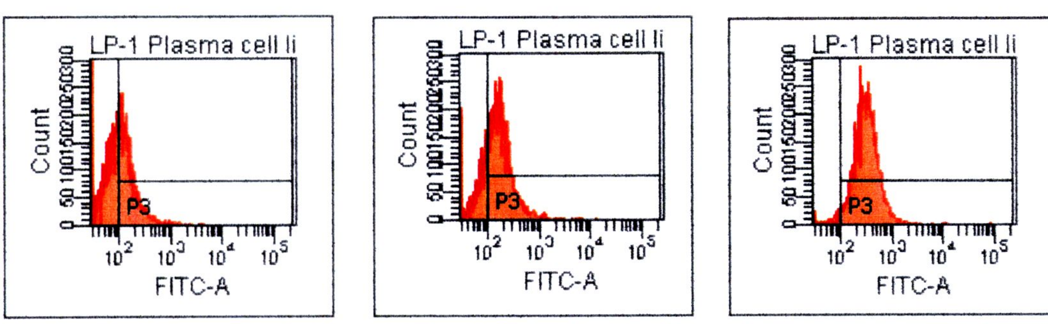
Unstained Conjugated control Blimp-1



	Unstained	Conjugated control	Blimp-1
% Cell	21.5	50.1	92.7
FITC Mean	251	301	376
FITC Median	131	229	322

Antibody 1:200

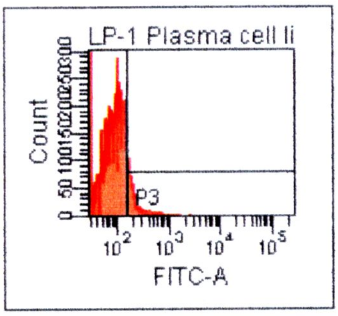
Unstained Conjugated control Blimp-1



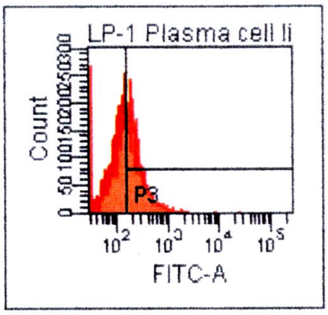
	Unstained	Conjugated control	Blimp-1
% Cell	21.5	47.5	90.1
FITC Mean	251	301	376
FITC Median	131	229	322

Comparison of permeabilizing agent
Cold Methanol

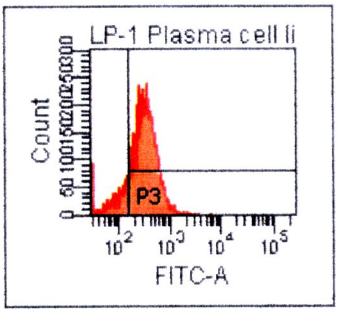
Unstained



Conjugated control



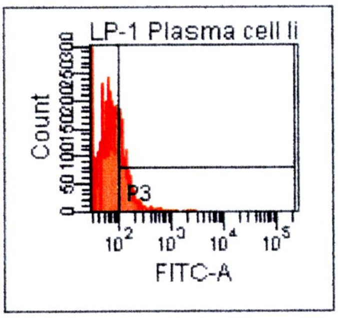
Blimp-1



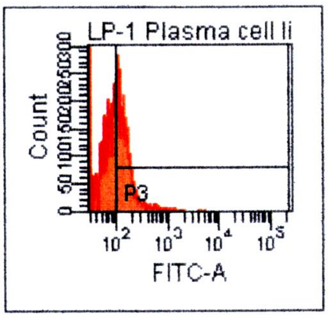
	Unstained	Conjugated control	Blimp-1
% Cell	9.2	41.1	82.4
FITC Mean	251	301	376
FITC Median	131	229	322

Triton x 100

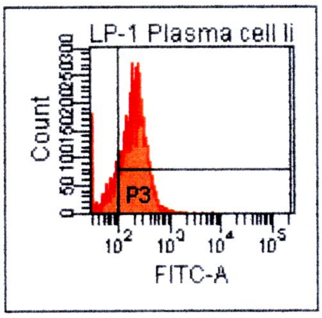
Unstained



Conjugated control



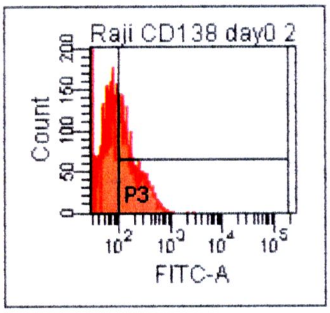
Blimp-1



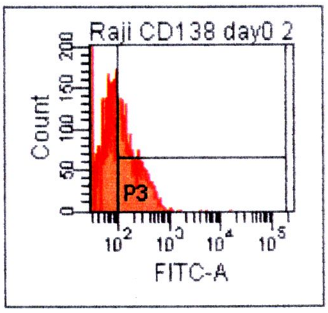
	Unstained	Conjugated control	Blimp-1
% Cell	18.9	39.0	80.8
FITC Mean	178	188	246
FITC Median	131	140	215

Untimulated Raji cell CD138 day 0

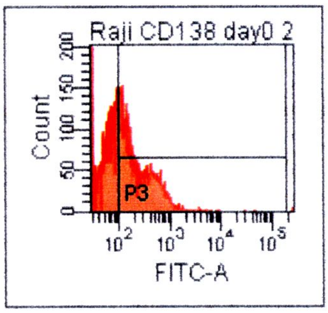
Unstained



Conjugated control



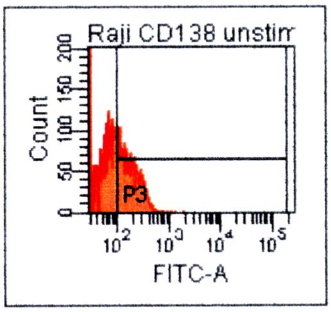
CD 138



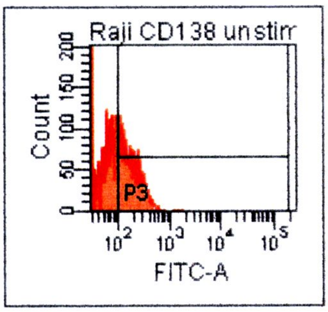
	Unstained	Conjugated control	CD 138
% Cell	39.7	41.0	51.7
FITC Mean	210	225	347
FITC Median	158	166	190

Unstimulated Raji cell CD138 day 3

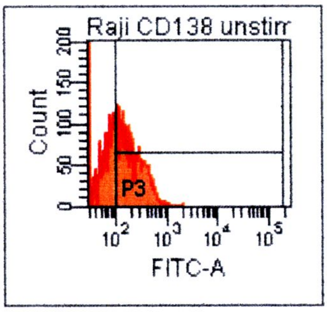
Unstained



Conjugated control



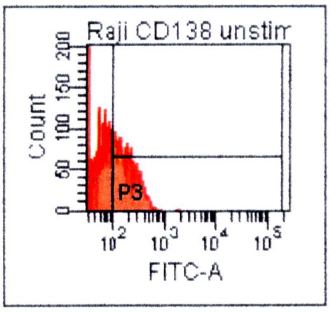
CD 138



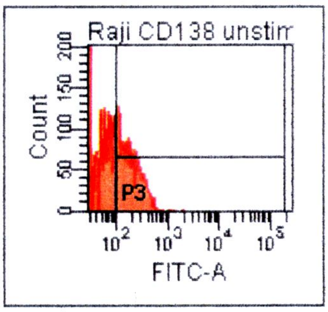
	Unstained	Conjugated control	CD 138
% Cell	42.2	43.4	57.6
FITC Mean	193	193	245
FITC Median	166	164	188

Unstimulated Raji cell CD138 day 6

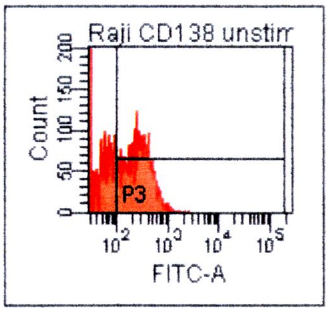
Unstained



Conjugated control



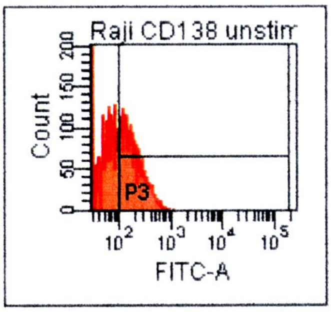
CD 138



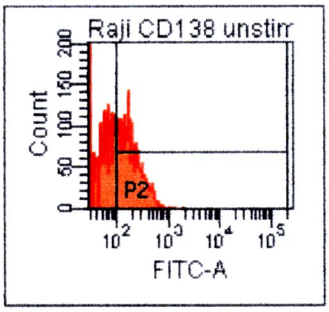
	Unstained	Conjugated control	CD 138
% Cell	38.2	41.5	56.4
FITC Mean	203	202	289
FITC Median	170	169	255

Unstimulated Raji cell CD138 day 9

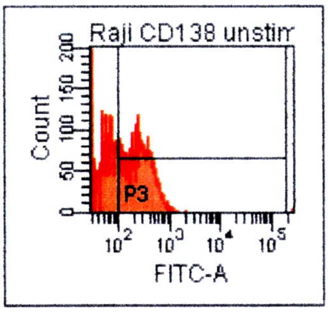
Unstained



Conjugated control



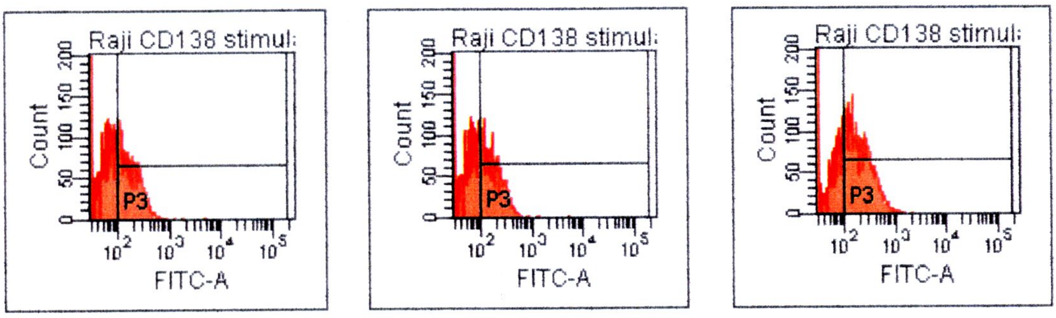
CD 138



	Unstained	Conjugated control	CD 138
% Cell	44.9	46.3	52.5
FITC Mean	204	215	294
FITC Median	164	176	251

Stimulated Raji cell CD138 day 3

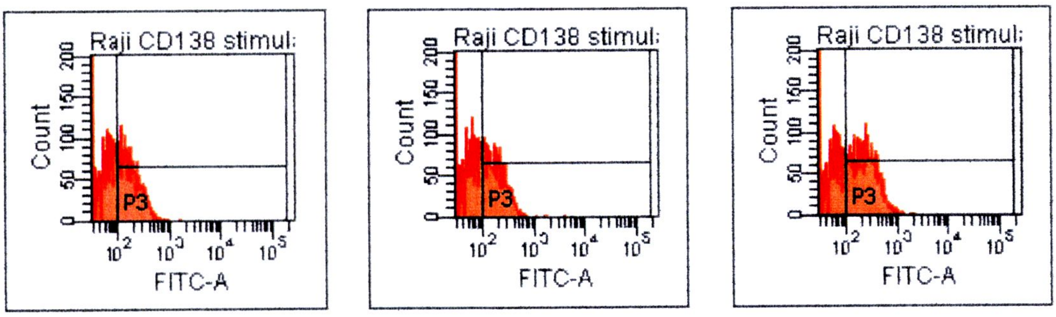
Unstained　　Conjugated control　　CD 138



	Unstained	Conjugated control	CD 138
% Cell	41.8	42.4	52.4
FITC Mean	192	189	132
FITC Median	164	160	190

Stimulated Raji cell CD138 day 6

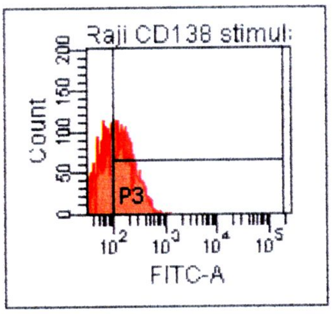
Unstained　　Conjugated control　　CD 138

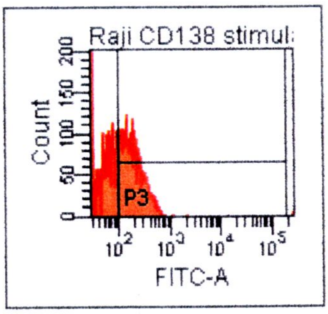


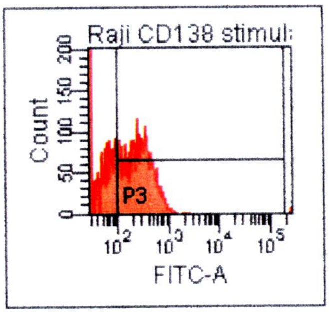
	Unstained	Conjugated control	CD 138
% Cell	40.3	40.4	54.7
FITC Mean	195	200	263
FITC Median	166	172	219

Stimulated Raji cell CD138 day 9

Unstained | Conjugated control | CD 138



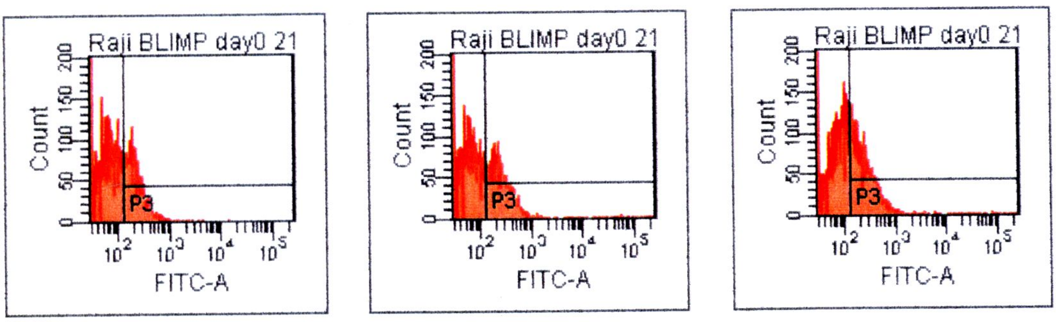




	Unstained	Conjugated control	CD 138
% Cell	46.1	50.0	60.4
FITC Mean	202	204	305
FITC Median	169	172	260

Unstimulated Raji cell Blimp-1 day 0

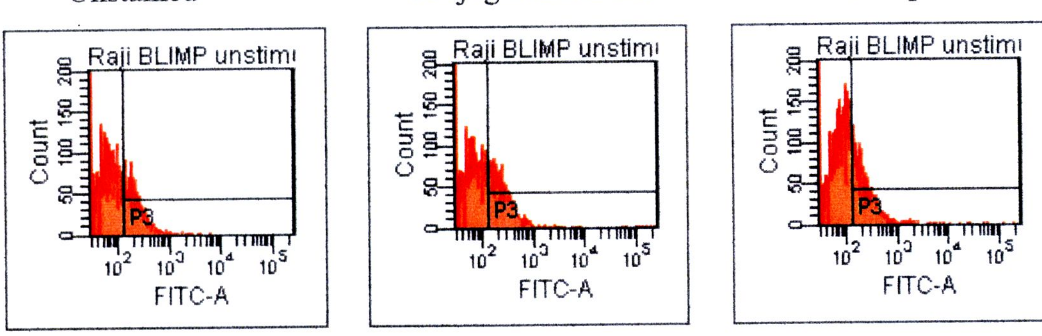
Unstained   Conjugated control   Blimp-1



	Unstained	Conjugated control	Blimp-1
% Cell	28.2	30.4	40.6
FITC Mean	253	2015	1437
FITC Median	198	233	223

Unstimulated Raji cell Blimp-1 day 3

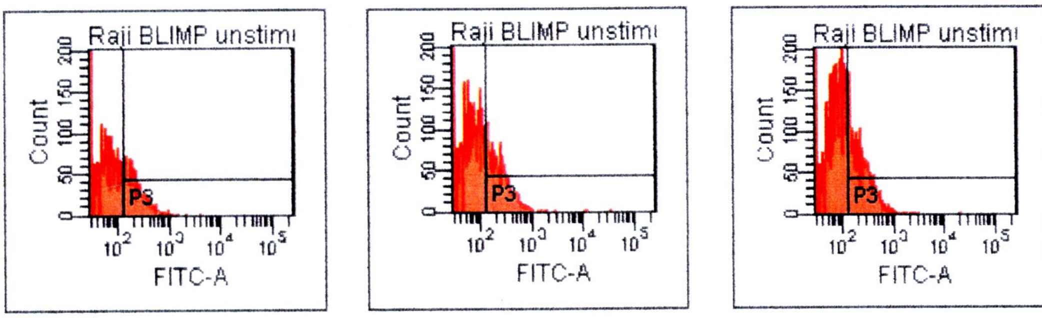
Unstained   Conjugated control   Blimp-1



	Unstained	Conjugated control	Blimp-1
% Cell	26.3	31.5	32.2
FITC Mean	269	915	631
FITC Median	202	221	198

Unstimulated Raji cell Blimp-1 day 6

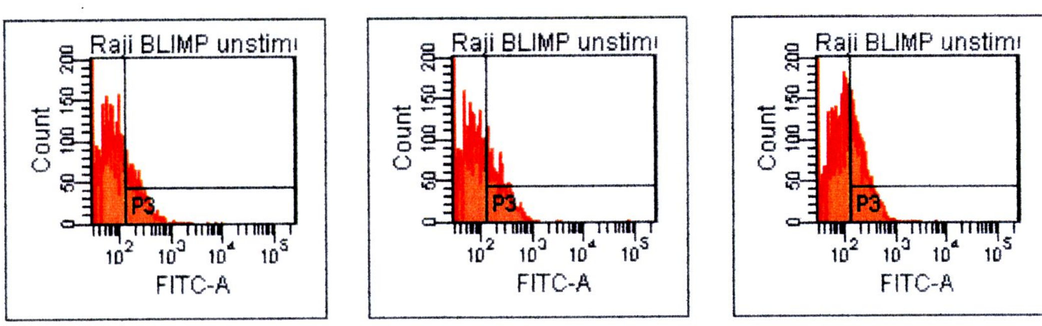
Unstained | Conjugated control | Blimp-1



	Unstained	Conjugated control	Blimp-1
% Cell	24.4	29.1	32.7
FITC Mean	254	285	271
FITC Median	198	211	213

Unstimulated Raji cell Blimp-1 day 9

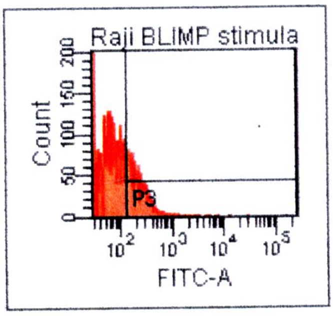
Unstained | Conjugated control | Blimp-1

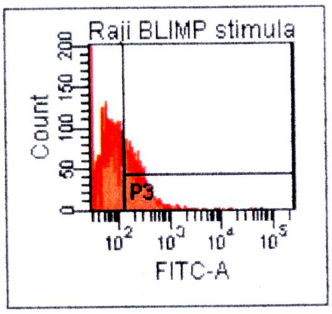


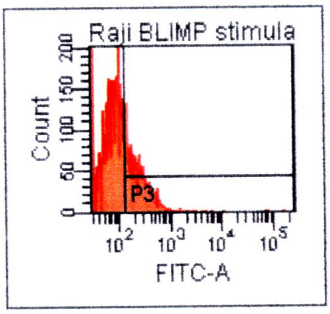
	Unstained	Conjugated control	Blimp-1
% Cell	22.6	27.2	37.8
FITC Mean	272	302	245
FITC Median	206	216	197

Stimulated Raji cell Blimp-1 day 3

Unstained | Conjugated control | Blimp-1



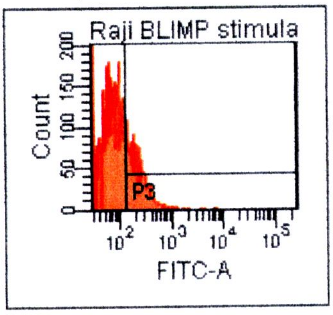


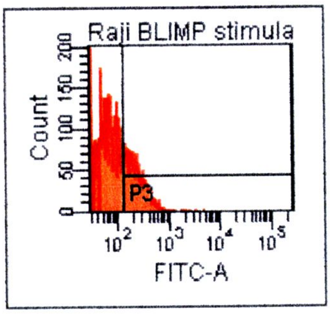


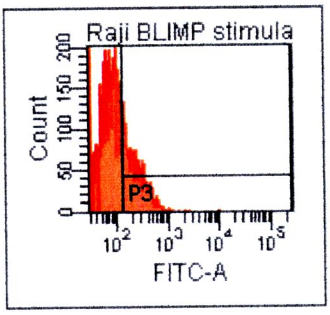
	Unstained	Conjugated control	Blimp-1
% Cell	24.2	32.5	28.5
FITC Mean	313	400	522
FITC Median	200	213	209

Stimulated Raji cell Blimp-1 day 6

Unstained | Conjugated control | Blimp-1



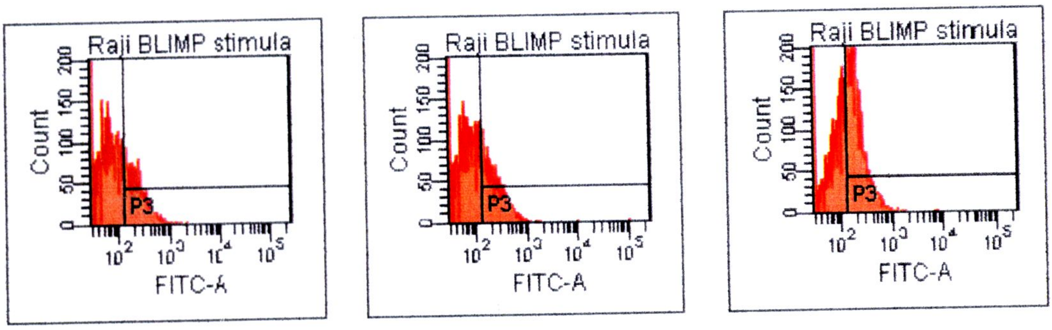




	Unstained	Conjugated control	Blimp-1
% Cell	25.2	25.1	27.8
FITC Mean	255	254	256
FITC Median	198	209	206

Stimulated Raji cell Blimp-1 day 9

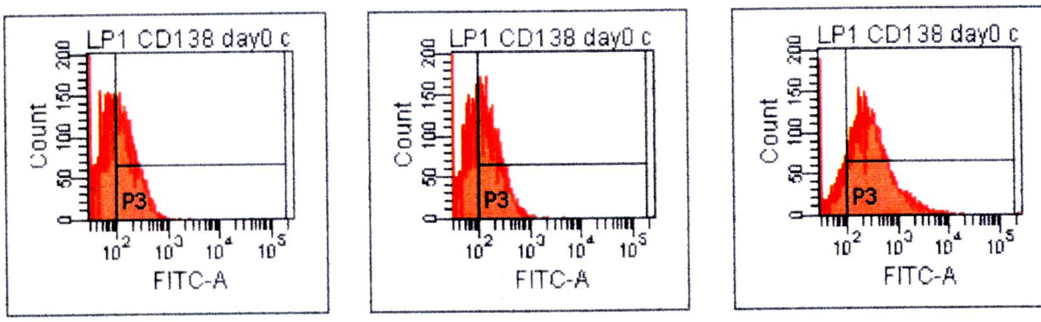
Unstained | Conjugated control | Blimp-1



	Unstained	Conjugated control	Blimp-1
% Cell	24.9	27.8	54.3
FITC Mean	246	316	228
FITC Median	207	209	189

LP-1 cell CD138 day 0

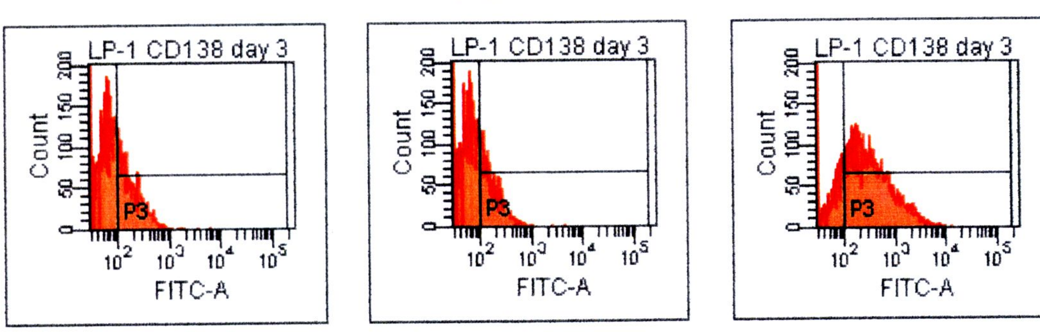
Unstained | Conjugated control | CD 138



	Unstained	Conjugated control	CD 138
% Cell	45.9	50.6	83.6
FITC Mean	202	207	511
FITC Median	163	166	284

LP-1 cell CD138 day 3

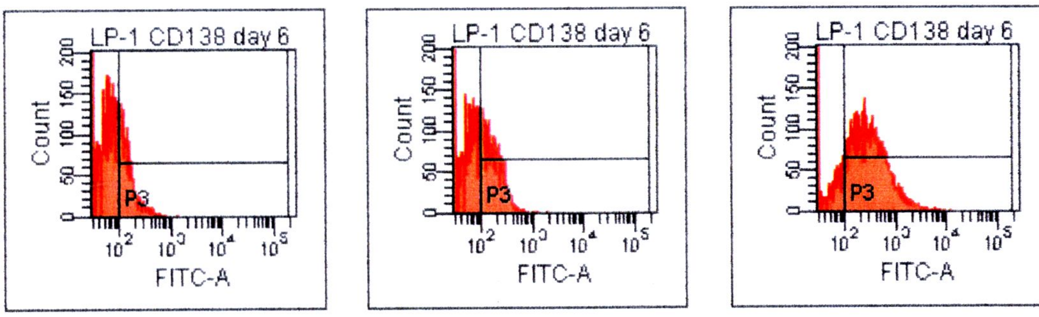
Unstained | Conjugated control | CD 138



	Unstained	Conjugated control	CD 138
% Cell	31.4	25.3	74.4
FITC Mean	215	185	588
FITC Median	161	143	303

LP-1 cell CD138 day 6

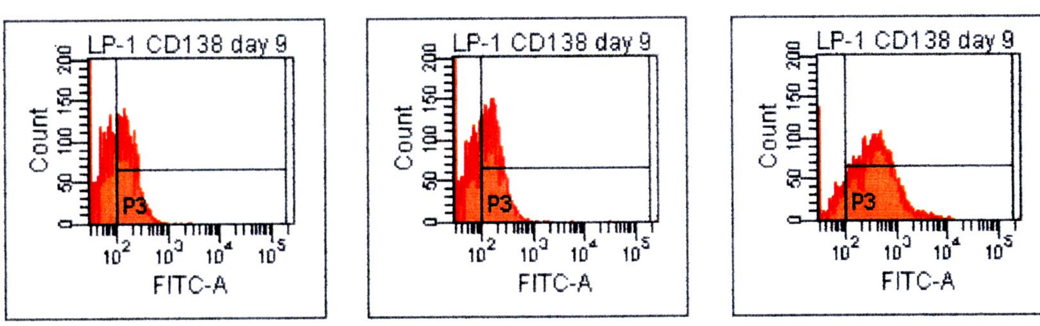
Unstained | Conjugated control | CD 138



	Unstained	Conjugated control	CD 138
% Cell	25.7	36.8	81.4
FITC Mean	155	184	449
FITC Median	128	158	289

LP-1 cell CD138 day 9

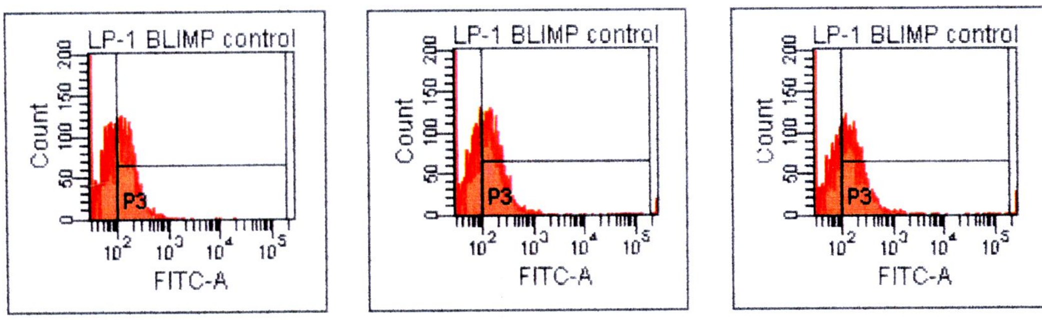
Unstained | Conjugated control | CD 138



	Unstained	Conjugated control	CD 138
% Cell	46.4	51.2	86.1
FITC Mean	186	200	595
FITC Median	159	162	382

LP-1 cell BLIMP-1 day 0

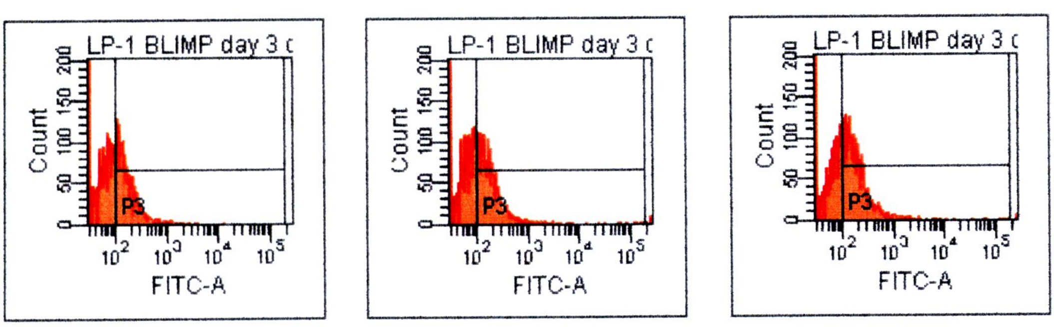
Unstained | Conjugated control | Blimp-1



	Unstained	Conjugated control	CD 138
% Cell	48.5	53.8	57.8
FITC Mean	199	658	666
FITC Median	153	161	166

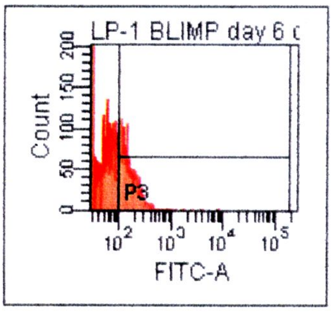
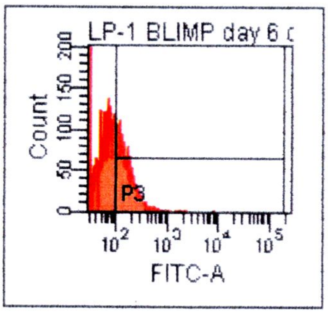
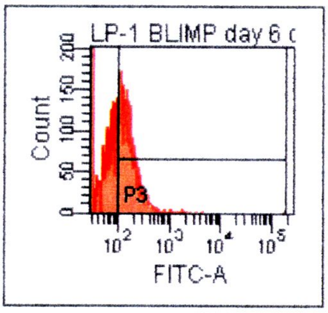
LP-1 cell BLIMP-1 day 3

Unstained | Conjugated control | Blimp-1



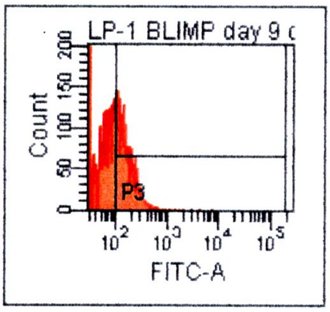
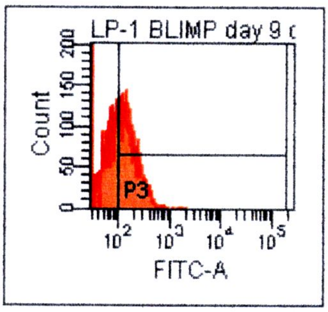
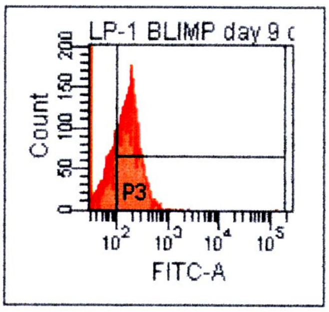
	Unstained	Conjugated control	Blimp-1
% Cell	44.1	48.6	56.5
FITC Mean	205	659	458
FITC Median	145	158	161

LP-1 cell BLIMP-1 day 6

Unstained	Conjugated control	Blimp-1
		

	Unstained	Conjugated control	Blimp-1
% Cell	32.5	33.8	50.4
FITC Mean	177	178	243
FITC Median	140	142	143

LP-1 cell BLIMP-1 day 9

Unstained	Conjugated control	Blimp-1
		

	Unstained	Conjugated control	Blimp-1
% Cell	41.1	51.7	70.5
FITC Mean	179	187	241
FITC Median	142	153	178

CURRICULUM VITAE

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