INFα induction

Reagent	Final concentration in cell culture	Stock conc	Vendor/provider
SLE-IgG from anti-snRNP+ serum	100 ug/ml	18,3 mg/ml (-80C)	Pfizer
Apoptotic U937	1 mill cells/ml	40 mill cells/ml (-80C)	Pfizer
R848	100 ng/ml	1 mg/ml, -20C	Enzo 420-038-M025
CpG-A ODN2216	0,25 ug/ml	303 ug/ml, -20C	InVivogen tlrl-2216
Pan IFNα ELISA kit			MabTech 3425-1A-20

Flow cytometry:

Marker	Fluorochrome	Vendor	Catalogue number	dilution
CD3	FITC	Biolegend	300406	1:25
CD303	PE	Miltenyi	130-090-511	1:10
HLA-DR	PerCP	Biolegend	307628	1:50
CD16	PECy7	Biolegend	302016	1:1667
CD86	AF647	Biolegend	305416	1:167
CD56	AF700	BD	557919	1:100
CD14	APCCy7	BD	557831	1:200
CD11c	BV421	Biolegend	562561	1:50
Aqua live/dead		InVitrogen	L34957	1:1000

Cell culture medium: RPMI, 1% PEST, 1% L-glutamine, 10% heat inactivated FCS
Ficoll-Paque PLUS GE Healthcare, #17-1440-03
BD stabilizing fixative #339860
Flow cytometry staining buffer: PBS + 0,05% FCS

Procedure:

1. Isolate PBMC from fresh sodium heparinized blood by Ficoll Paque separation
2. Stain 1 mill cells for FACS analysis
3. Dilute cells to $5x10^6$ cells/ml in cell culture medium
4. Plate the cells in 96 well U-bottomed wells, 100 µl/well, triplicates
5. Add probes in triplicate in 50 µl/well (4x final concentration, diluted in medium) and incubate 30 min at 37C. Add 50 µl medium to wells without probe.
6. Add SLE-IgG mixed with U937 in 50 µl/well (4x final concentration, diluted in medium) making the total volume per well 200 µl. Include R848 and ODN2216 (without probe) as positive controls. Add 50 µl medium to wells without stimulus.
7. Add PBS to outer wells
8. Incubate 24h 37C

9. Collect supernatants
10. Freeze the supernatants at -20C until analysis of IFNα concentration using ELISA