

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A01+2A02/2018

UK NEQAS for H&I Scheme 2A - Cytotoxic Crossmatching

DESPATCHED ON 20TH MARCH 2018

Lab No.	Cells used			Immunobeaded cells used?		Complement Source	DTT addition		DTT Concentration	Volumes				Incubation Times				Visualisation of dead cells	Modifications to the standard NIH CDCXM method	Comments		
	PBL	T-cells	B-cells	T-cells	B-cells		Sera	Cells		DTT	Sera	Cells	Comp	DTT	B-cell	Pre Comp T-cell	B-cell				Post Comp T-cell	B-cell
9	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	5	95	1	5	30	30	30	30	30	Fluoroquench				
11	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.008M	1	1	1	5	30	30	45	45	90	90	Fluoroquench			
12	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	60	EB/AO			
15	Yes	Yes	Yes	Yes	Yes	TCS Biosciences	Yes	0.02M	1	2	1	5	30	30	60	60	60	60	Fluoroquench			
20	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	60	60	EB/AO			
23	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	40	40	40	40	EB/AO			
24	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	4:36	1	1	5	30	30	45	45	90	90	Fluoroquench			
25	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	9:81	1	1	5	30	30	30	30	45	45	Fluoroquench			
28	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	45	45	EB/AO			
34	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	90	EB/AO			
38	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	60	60	60	60	60	60	EB/AO			
39	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	0.1	1	1	5	10	10	45	45	90	90	Fluoroquench			
41	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	45	45	45	45	EB/AO			
42	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	12mg/ml	1	1	1	5	30	30	60	60	120	60	Fluoroquench			
45	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	60	30	90	75	EB/AO			
51	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	60	60	60	60	Fluoroquench			
54	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	60	60	PI/AO			
58	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	1	2	1	5	45	45	60	60	90	90	Fluoroquench			
101	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.05M					45	45					Fluoroquench			
112	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1M	0.1	0.9	1	5	30		60		120		Stain Fix			
114	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.05M	10	90	1	5			35	45	75	90	Fluoroquench	AMOS modification	DTT treatment of the sera is done in a separate tube 4µl DTT and 36µl sera. Cells extracted with Rosettesep	
116	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05	1-5	1-1	2-4	30	30	20	20	30	30	30	EB/AO			
117	Yes	Yes	Yes	Yes	Yes	Life Technology	Yes	1%	1	1	1	5	30		30		60		Fluoroquench			
119	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.005M	1	1	1	6			30	30	60	60	Eosin			
120	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5			30	30	60	60	Fluoroquench			
128	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.01M	1	1	1	5			45	90	90	60	Eosin		Sera treatment with DTT prior to CDC incubation: 2µl DTT 0.1M + 38µl serum - incubation 30mn 37°C	
133	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.01M	1	1	1	5	90	90	30	30	60	60	Fluoroquench		We resuspend the cell pellet with DTT in PBS (0.01M) and use 1 microliter of cell suspension per well	
136	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.05M	1	1	1	5			35	35	75	75	EB/AO			
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	20	20	30	30	60	60	Fluoroquench	Washes		
143	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	45		30	60	60	60	Eosin			
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.005M	1	19	1	5	30	30	45	45	45	45	EB/AO			
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.005M	1	19	1	5	30	30	30	30	60	60	Fluoroquench			
149	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.5	0.5	1	5			60		60		Eosin				
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5			30	30	60	60	Eosin			
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	10	90	1	5	45	45	30	30	60	120	Fluoroquench	AHG incubation during 3 minutes		
159	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.01M	10	90	1	5	45	45	60	60	60	60	Fluoroquench			
162	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	10	90	1	5	45	45	60	60	60	60	Fluoroquench			
163	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	30	30	60	60	Fluoroquench			
169	Yes	Yes	Yes	Yes	Yes	In-house	Yes	50mM	0.1	1	1	5			30	30	60	60	Fluoroquench			
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5			30	30	45	45	Fluoroquench			
186	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	5mM	3	57	1	6	30	30	30	30	60	60	Fluoroquench			
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1mg/l	2	2	2	5	30	30	60	60	30	30	EB/AO			
190	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1mg/l	1	19	1	5	30	30	30	30	60	60	Fluoroquench			
193	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	0.05	0.95	1	5	30	30	30	30	60	60	Stain Fix			
194	Yes	Yes	Yes	Yes	Yes	In-house	Yes	5%	1	1	1	6	15	15	30	30	45	45	Fluoroquench			
195	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	2	1, 2	1	5	15	15	30	30	60	60	Fluoroquench			
197	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1/20	1	1	1	5	15	15	45	45	90	90	Fluoroquench			
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.5	0.5	0.5	3.3			30	30	45	45	Fluoroquench			
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.0015M	1	2	1	5	10	10	30	30	60	60	Fluoroquench	CDC with AGH on T-lymphocytes during 2 minutes		
209	Yes	Yes	Yes	Yes	Yes	One Lambda, Ingen	Yes	0.0015M	10	90	1	5			30	60	30	60	PI/AO			
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	5	1	1	6			30	60	30	60	EB/AO			
223	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	1/10	5	45	1	5			30	30	60	60	Fluoroquench	15m incubation of serum with DTT at 37°C before cell/serum deposit		
227	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/10	5	45	1	5			30	30	60	60	Fluoroquench			
232	Yes	Yes	Yes	Yes	Yes	Biotech	Yes	0.001M	1	1	0.5	2.5			30	45	60	60	EB/AO			
235	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.001M	1	1	1	5	30		30		60		Eosin			
238	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1/20	2	38	1	5	30	30	30	45	60	90	Fluoroquench			
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	1	1	1	5			30	60	60	120	Fluoroquench			
245	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.1M%	5	95	1, 2	5	30	30	30	30	60	45	EB/AO			
252	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	0.1	1	1	5	30	30	30	30	60	60	Fluoroquench			
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/10	1	1	1	5	30	30	30	30	60	60	Fluoroquench			
262	Yes	Yes	Yes	Yes	Yes	Life Technologies	Yes	1/2	1	1	1	5			30	60	60	90	Eosin			
268	Yes	Yes	Yes	Yes	Yes	Gentrack	Yes	1/10	3	27	1	5			30	30	60	90	Fluoroquench	XM LCT with AHG on T-cells		
284	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M/L	1	19	1	5	35	35	40	40	60	60	EB/AO	T-cells tested with and without AHG		
292	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.015M	1	1	1	3	15	15	30	30	60	60	Fluoroquench			
293	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.05M	50	450	1	6	60	60	60	60	120	120	Eosin			
294	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M/ml	1	19	1	3	15	15	30	30	30	30	Fluoroquench			
297	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.001Mol/ml	1	1	1	6	30	30	30	30	60	60	Fluoroquench			
311	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	1	1	5	30	30	30	30	60	60	Fluoroquench			
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	4	76	1	5	15	15	30	30	50	50	Fluoroquench			
321	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	2	38	1	7			30	30	60	60	Fluoroquench			
351																						

Denotes 2017 methodology/No methodology received

EB - ethidium bromide
AO - acridine orange
PI - propidium iodide
Hb - haemoglobin
CFDA - carboxy-fluorescein diacetate