

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A05+2A06/2018

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

DESPATCHED ON 11TH SEPTEMBER 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
9	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	5	95	1	5	30	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	60	60	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	30	60	120			Stain Fix		
114	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	1/4	1	1	1	5	30	30	35	45	75	90	Fluoroquench		
116	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05	1-5	1	2-4	30	30	20	20	30	30		EB/AO	AMOS modification	DTT treatment of the sera is done in a separate tube 4µl DTT and 36µl sera. Cells extracted with Rosettesep
117	Yes	Yes	Yes	Yes	Yes	Life Technology	Yes	1%	1	1	1	5	30	30	30	60	60		Eosin		
119	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.005M	1	1	1	6	30	30	30	60	60		Fluoroquench		
120	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	45	90	90		Eosin		
128	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.01M	1	1	1	5	90	90	30	30	60	60	Fluoroquench		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	35	35	75	75			EB/AO		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	20	20	30	30	60	60	Fluoroquench	Washes	
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	45	45	30	60	60		Eosin		
143	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	1	1	1	5	30	30	30	30	60	60	Fluoroquench		
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	1:10	1	1	1	5	45	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	60	60	60			Eosin		
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:4	1	1	1	5	180	180	60	60	120	120	Fluoroquench		
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	10	90	1	5	45	45	30	30	60	60	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	30	30	60	60	Fluoroquench		
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	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	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	Biorad	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	1	5	30	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	1/10	10	90	1	5	30	30	60	30	60		PI/AO		
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	5	1	1	6	30	30	60	60	60		EB/AO		
223	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	1/10	5	45	1	5	30	30	30	30	60	60	Fluoroquench	15m incubation of serum with DTT at 37°C before cell/serum deposite	
227	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/10	5	45	1	5	30	30	60	60	60		Fluoroquench		
229	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.001M	1	1	1	5	30	30	45	60	120		Fluoroquench		
232	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/20	2	38	1	5	30	30	30	45	60	90	Fluoroquench		
235	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/20	1	1	1	5	30	30	30	60	120		Fluoroquench		
238	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M%	5	95	1,2	5	30	30	30	30	60	45	EB/AO		
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M%	0.1	1	1	5	30	30	30	30	60	60	Fluoroquench		
245	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.1M%	1	1	1	5	30	30	30	30	60	60	Fluoroquench		
252	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	1	1	1	5	30	30	30	30	60	60	Fluoroquench		
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M	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	30	60	60	90		Eosin		
268	Yes	Yes	Yes	Yes	Yes	Gentrack	Yes	1/10	3	27	1	5	30	30	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	3	60	60	60	60	120	120	Eosin		
294	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1Mol	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	3	57	1	5	30	30	30	30	60	60	Fluoroquench		
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	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	30	60	60	Fluoroquench			
351	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	10	90	1	5	30	30	30	60	30	60	Fluoroquench		
411	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	10	90	1	5	30	30	30	60	30	60	Fluoroquench		

Denotes 2017 methodology/No methodology received

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