

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A07+2A08/2017

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

DESPATCHED ON 18th July 2017

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		0.05M	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			
14	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		10%	0.1	0.9	1	5	10	10	45	45	75	75	Fluoroquench			
15	Yes	Yes	Yes	Yes	Yes	Cedarlane	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			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		1:10	1	1	1	5	30	30	30	30	45	45	Fluoroquench			
28	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	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			
35	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	10	70		5	10	60	60	60	60	90	Fluoroquench			
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			0.1	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	TSC Biosciences	Yes	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			1	1	1	5	0	0	60	60	60	60	Fluoroquench			
54	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	1	1	5	60	60	60	60	60	60	PI/AO			
58	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	2	1	5	45	45	60	60	90	90	Fluoroquench			
62	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes													EB/AO	One hour incubation with cells, two hours with complement		
101	Yes	Yes	Yes	Yes	Yes	BAG	Yes		0.05M					45	45					Fluoroquench			
114	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1M	0.1	0.9	1	5	30		60		120		Stain Fix			
116	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes		0.05M	0.1	1	1	5	30	30	30	60	30	60	Fluoroquench	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	Cedarlane	Yes		0.05		1-5	1	2-4	30	30	20	20	30	30	EB/AO			
119	Yes	Yes	Yes	Yes	Yes	Life Technology Biorad	Yes			1	1	1	5	30		30		60		Eosin, Fluoroquench			
120	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.005M	1	1	1	6	30	30	60	60	60	60	Fluoroquench		Sera treatment with DTT prior to CDC incubation: 2µl DTT 0.1M + 38µl serum - incubation 30mn 37c	
128	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes			1	1	1	5	90	90	30	30	60	60	Eosin		We resuspend the cell pellet with DTT in PBS (0.01M) and use 1 microliter of cell suspension per well	
133	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		0.01M	1	1	1	5	60	60	60	60	60	60	Fluoroquench			
136	Yes	Yes	Yes	Yes	Yes	In-house	Yes			1	1	1	5	45	45	60	60	60	60	EB/AO			
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.05M	5	45	1	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	30	60	60	60	Eosin			
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes			1	19			30	30	45	45	45	45	EB/AO			
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes		0.005M	1	19			30	30	30	30	60	60	Fluoroquench			
149	Yes	Yes	Yes	Yes	Yes	Commercial Cedarlane	Yes			0.5	0.5	1	5	30	30	60	60	60	60	Eosin			
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes							60	60	120	120	120	120	Fluoroquench			
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	Yes	0.005M	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		0.05M	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	Invitrogen	Yes		5mM	1	19	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			1	19	1	5	30	30	30	30	60	60	Fluoroquench			
193	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1mg/l	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	Invitrogen	Yes		0.1M	2	1,2	1	5	15	15	30	30	60	60	Fluoroquench			
<b>197</b>																							
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes			1/20	1	1	5	15	15	45	45	90	90	Fluoroquench			
205	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes			25	75			90						30	60		
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	Yes		0.01M	0.5	0.5	1	3.3		30	30	45	45	Fluoroquench			
209	Yes	Yes	Yes	Yes	Yes	One Lambda, Ingen	Yes			0.0015M	1	2	1	5	10	10	30	30	60	60	Fluoroquench	CDC with AGH on T-lymphocytes during 2 minutes	
<b>212</b>																							
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes			10	90	1	5			30		60		PI/AO			
223	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes							45	45	60	60	60	60	EB/AO			
227	Yes	Yes	Yes	Yes	Yes	Immucor	Yes		0.5M	0.05	0.95	1	6	30	30	60	60	60	60	Fluoroquench	15mn incubation of serum with DTT at 37c before cell/serum deposite		
229	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/9	0.3	2.7	1	5	30	30	60	60	60	60	Fluoroquench			
232	Yes	Yes	Yes	Yes	Yes	Biotest	Yes				0.5	0.5	2.5			45		60		EB/AO			
235	Yes	Yes	Yes	Yes	Yes	Biorad	Yes		0.001M	1	1	1	5	30	30	30	30	60	60	Eosin			
238	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes		1/20	2	38	5	5	30	30	30	45	60	90	Fluoroquench			
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes			5	1	1	5	30	30	30	30	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			0.1	1	1	5	30	30	30	30	60	60	Fluoroquench			
259	Yes	Yes	Yes	Yes	Yes	Biorad	Yes							30	30	30	30	60	60	PI/AO			
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes			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	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			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	5	1	1	5	30	30	30	30	60	60	Fluoroquench			
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes		1%	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	30	60	60	Fluoroquench			

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