

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A09+2A10/2017

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

DESPATCHED ON 12th September 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	30	Fluoroquench			
11	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.009M	1	1	1	5	30	30	45	45	90	90	90	Fluoroquench			
12	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	60	60	EB/AO			
14	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	10%	0.1	0.9	1	5	10	10	45	45	75	75	75	Fluoroquench			
15	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.02M	1	2	1	5	30	30	60	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	60	EB/AO			
23	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1	1	1	5	30	30	40	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	90	Fluoroquench			
25	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	1	5	30	30	30	30	45	45	45	Fluoroquench			
28	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	45	45	45	EB/AO			
34	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	90	90	EB/AO			
35	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	10	70		5	10	10	45	60	90	90	90	Fluoroquench			
38	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	60	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	90	Fluoroquench			
41	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	45	45	45	45	45	EB/AO			
42	Yes	Yes	Yes	Yes	Yes	TSC Biosciences	Yes	12mg/ml	1	1	1	5	30	30	60	60	120	60	60	Fluoroquench			
45	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	60	30	90	75	75	EB/AO			
51	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1	1	1	5	0	0	60	60	60	60	60	Fluoroquench			
54	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1	1	1	5	60	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	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			
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	0.1	1	1	5	30		60	60	30	60	60	Fluoroquench			
116	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05		1.5	1	2.4	30	30	20	20	30	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													Eosin, Fluoroquench			
119	Yes	Yes	Yes	Yes	Yes	Biorad	Yes		1	1	1	5	30		30		60			Eosin			
120	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	6	30	30	60	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	45		90		90			Eosin			
133	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.01M	1	1	1	5	90	90	30	30	60	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		1	1	1	5	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	60	Fluoroquench	Washes		
143	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	45		30		60			Eosin			
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes								45	45	45	45	45	EB/AO			
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.005M	1	19		5	30	30	30	30	60	60	60	Fluoroquench			
149	Yes	Yes	Yes	Yes	Yes	Commercial	Yes		0.5	0.5	1	5	30		60		60			Eosin			
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes								30	30	60	60	60	Eosin			
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	60		60		120	120	120	Fluoroquench			
159	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.05M	10	90	1	5	45	45	30	30	60	60	60	Fluoroquench	AHG incubation during 3 minutes		
162	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	10	90	1	5	45	45	60	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	60	Fluoroquench			
169	Yes	Yes	Yes	Yes	Yes	In-house	Yes	50mM	0.1	1	1	5	30		30		30		60	Fluoroquench			
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30		30		30		45	45	45	45	
186	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	5mM	1	19	1	6	30	30	30	30	60	60	60	Fluoroquench			
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		2	2	2	5	30	30	60	60	30	30	30	EB/AO			
190	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	1mg/l	1	19	1	5	30	30	30	30	60	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	60	Fluoroquench			
194	Yes	Yes	Yes	Yes	Yes	In-house	Yes	5%	1	1	1	6	15	15	30	30	45	45	45	Fluoroquench			
195	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.1M	2	1.2	1	5	15	15	30	30	60	60	60	Fluoroquench			
197																							
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/20	1	1	5	15	15	45	45	90	90	90	Fluoroquench			
205	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		25	75					30		60		60	Fluoroquench			
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.5	0.5	0.5	3.3	30		30		45	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	60	Fluoroquench	CDC with AGH on T-lymphocytes during 2 minutes		
212																							
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		10	90	1	5	30		30		60			PI/AO			
223	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes								45	45	60	60	60	EB/AO			
227	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	5	1	1	6	30	30	60	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	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		60			Eosin			
238	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	1/20	2	38		5	30	30	30	45	60	90	90	Fluoroquench			
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes			1	1	5	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	45	EB/AO			
252	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		0.1	1	1	5	30	30	30	30	60	60	60	Fluoroquench			
259	Yes	Yes	Yes	Yes	Yes	Biorad	Yes			1	1	5	30	30	30	30	60	60	60	PI/AO			
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		1	1	1	5	30	30	30	30	60	60	60	Fluoroquench			
262	Yes	Yes	Yes	Yes	Yes	Life Technologies	Yes	1/2	1	1	1	5	30		30		60		90	Eosin			
268	Yes	Yes	Yes	Yes	Yes	Gentrack	Yes	1/10	3	27	1	5	30		30		30		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	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	60	Fluoroquench			
293	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.05M	50	450			60	60	60	60	120	120	120	Eosin			
294	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1Mol	1	19	1	3	15	15	30	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	60	Fluoroquench			
311	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	1	1	5	30	30	30	30	60	60	60	Fluoroquench			
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	1%	4	76	1	5	15	15	30	30	50	50	50	Fluoroquench			
321	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	2	38	1	7	30		30		60		60	Fluoroquench			

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