

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A09+2A10/2016

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

DESPATCHED ON 20TH SEPTEMBER 2016

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				B-cell	Post Comp
9	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	5	95	1	5	30	30	30	30	30	Fluorouquench			
11	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.025M	1	1	1	5	30	30	45	45	90	Fluorouquench			
12	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	EB/AO			
14	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	10%	0.1	0.9	1	5	10	10	45	45	75	Fluorouquench			
15	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.02M	1	2	1	5	30	30	60	60	60	Fluorouquench			
20	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	60	EB/AO			
23	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	4:36	1	1	5	30	30	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	Fluorouquench			
25	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	1	5	30	30	30	30	45	Fluorouquench			
28	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	45	EB/AO			
34	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	30	30	30	30	90	EB/AO			
35	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	10	70	1	5	10	60	45	60	90	Fluorouquench			
38	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1	1	1	5	60	60	60	60	60	EB/AO			
39	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.1	1	1	5	10	10	45	45	90	Fluorouquench			
41	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	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	Fluorouquench			
45	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	60	30	90	EB/AO			
51	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	0	0	60	60	60	Fluorouquench			
54	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	60	60	60	60	60	PI/AO			
58	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	2	1	5	45	45	60	60	90	Fluorouquench			
62	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	2	1	5	45	45	60	60	90	EB/AO			
101	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.05M					45	45				Fluorouquench		One hour incubation with cells, two hours with complement	
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	30	30	30	60	Fluorouquench			
116	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05		1-5	1	2-4	30	30	20	20	30	EB/AO		AMOS modification	
117	Yes	Yes	Yes	Yes	Yes	Life Technology	Yes	0.05M					60				120	Eosin, Fluorouquench		DTT treatment of the sera is done in a separate tube 4µl DTT and 36µl sera. Cells extracted with Rosettesep	
119	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.005M	1	1	1	5	30		30	60		Eosin			
120	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30	30	60	60	60	Fluorouquench		Sera treatment with DTT prior to CDC incubation: 2µl DTT 0.1M + 38µl serum - incubation 30mn 37°C	
128	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.001M	1	1	1	5	90	90	30	30	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	Fluorouquench			
136	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.05M	5	45	1	5	20	20	30	30	60	EB/AO			
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	5	45	1	5	20	20	30	30	60	Fluorouquench			
143	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	45		30	60	60	Eosin		Washes	
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.005M	1	19	1	5	30	30	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	Fluorouquench			
149	Yes	Yes	Yes	Yes	Yes	Commercial	Yes	0.005M	0.5	0.5	1	5	30		60	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	0.005M	1	1	1	5	60	60	60	120	120	Fluorouquench			
159	Yes	Yes	Yes	Yes	Yes	In-House	Yes	0.05M	10	90	1	5	45	45	30	30	60	Fluorouquench		AHG incubation during 3 minutes	
162	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	10	90	1	5	45	45	60	60	60	Fluorouquench			
163	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	30	30	60	Fluorouquench			
169	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	50mM	1	1	1	5	30		35	45	75	75	Fluorouquench		
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	30		30	45	45	Fluorouquench			
186	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	5mM	1	19	1	6	30	30	30	30	60	Fluorouquench			
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	2	2	2	5	30	30	60	60	60	60	EB/AO			
190	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1mg/l	1	19	1	5	30	30	30	60	60	Fluorouquench			
193	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1mg/l	0.05	0.95	1	5	30	30	30	30	60	Stain Fix			
194	Yes	Yes	Yes	Yes	Yes	In-House	Yes	5%	1	1	1	6	15	15	30	30	45	45	Fluorouquench		
195	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.1M	2	1, 2	1	5	15	15	30	30	60	Fluorouquench			
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	1/20	1	1	5	15	15	45	45	90	Fluorouquench			
205	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.01M	10	90	1	5	90	90	30	30	60	Fluorouquench			
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.5	0.5, 1	0.5	3.3	30		30	45	45	Fluorouquench			
209	Yes	Yes	Yes	Yes	Yes	One Lambda, Ingen	Yes	0.0015M	1	2	1	5	10	10	30	30	60	Fluorouquench		CDC with AGH on T-lymphocytes during 2 minutes	
212	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/10	10	90	1	5	30		30	60	60	PI/AO			
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	1/10	10	90	1	5	30		45	45	60	EB/AO			
223	Yes	Yes	Yes	Yes	Yes	Immucor	Yes	0.5M	0.05	0.95	1	6	30		30	60	60	Fluorouquench		15mn incubation of serum with DTT at 37°C before cell/serum deposit	
227	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1/9	0.3	2.7	1	5	30		30	60	60	Fluorouquench			
229	Yes	Yes	Yes	Yes	Yes	Biotest	Yes	0.001M	1	0.5	0.5	2.5	45		60	60	60	EB/AO			
232	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.001M	1	1	1	5	30		30	60	60	Eosin			
235	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	1/20	2	38	1	5	30		30	45	60	90	Fluorouquench		
238	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.001M	5	1	1	5	30		30	60	120	Fluorouquench			
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.001M	5	1	1	5	30		30	60	60	Fluorouquench			
245	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.1M%	5	95	1, 2	5	30		30	30	60	45	EB/AO		
252	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M%	0.1	1	1	5	30		30	60	60	Fluorouquench			
259	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.1M%	0.1	1	1	5	30		30	60	60	PI/AO			
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.1M%	1	1	1	5	30		30	60	60	Fluorouquench			
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	60	90	Fluorouquench			
284	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M/L	1	19	1	5	35	35	40	40	60	EB/AO		XM LCT with AHG on T-cells	
292	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.015M	1	1	1	3	15	15	30	30	60	Fluorouquench		T-cells tested with and without AHG	
293	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.05M	50	450	1	5	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	Fluorouquench			
297	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.001Mol/ml	1	1	1	6	30		30	30	60	Fluorouquench			
311	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	1	1	5	30		30	30	60	Fluorouquench			
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	1%	4	76	1	5	15	15	30	30	50	Fluorouquench			
321	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	2	38	1	7	30		30	60	60	Fluorouquench			

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