

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 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	0.025M	1	1	1	5	30	30	45	45	90	Fluoroquench		
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	Fluoroquench		
15	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.02M	1	2	1	5	30	30	60	60	60	Fluoroquench		
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		1	1	1	5		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	Fluoroquench		
25	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	1	5	30	30	30	30	45	Fluoroquench		
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		5	10		45	90	Fluoroquench			
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	0.1M	0.1	1	1	5	10	10	45	45	90	Fluoroquench		
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	Fluoroquench		
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		1	1	1	5	0	0	60	60	60	Fluoroquench		
54	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1	1	1	5			60	60	60	PI/AO		
58	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1	2	1	5	45	45	60	60	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	30	30	60	30	Fluoroquench		
116	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05		1-5	1	2-4	30	30	20	20	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								60		120	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	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		Eosin		
133	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.01M	1	1	1	5	90	90	30	30	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		EB/AO		
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	5	45	1	5	20	20	30	30	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	EB/AO		
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.005M	1	19			30	30	30	30	60	Fluoroquench		
149	Yes	Yes	Yes	Yes	Yes	Commercial	Yes		0.5	0.5	1	5		60		60		Eosin		
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes							30	30	60	60	Eosin		
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5		60	60	120	120	Fluoroquench		
159	Yes	Yes	Yes	Yes	Yes	In-House	Yes	0.05M	10	90	1	5	45	45	30	30	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	Fluoroquench		
163	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	1	1	1	5	30	30	30	30	60	Fluoroquench		
169	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	50mM	1	1	1	5		35	45	75	75	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	Invitrogen	Yes	5mM	1	19	1	6	30	30	30	30	60	Fluoroquench		
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		2	2	2	5	30	30	60	60	30	EB/AO		
190	Yes	Yes	Yes	Yes	Yes	Servbio	Yes		1	19	1	5	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	Stain Fix		
194	Yes	Yes	Yes	Yes	Yes	In-House	Yes	5%	1	1	1	6	15	15	30	30	45	Fluoroquench		
195	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.1M	2	1, 2	1	5	15	15	30	30	60	Fluoroquench		
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/20	1	1	5	15	15	45	45	90	Fluoroquench		
205	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		10	90							30	Fluoroquench		
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.5	0.5, 1	0.5	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	Fluoroquench		CDC with AGH on T-lymphocytes during 2 minutes
212	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		10	90	1	5		30		60		PI/AO		
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes							45	45	60	60	EB/AO		
223	Yes	Yes	Yes	Yes	Yes	Immucor	Yes	0.5M	0.05	0.95	1	6		30	30	60	60	Fluoroquench		
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	Fluoroquench		
239	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		5	1	1	5		30	60	120		Fluoroquench		
245	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.1M%	5	95	1, 2	5	30	30	30	30	60	EB/AO		
252	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		0.1	1	1	5	30	30			60	Fluoroquench		
259	Yes	Yes	Yes	Yes	Yes	Biorad	Yes			1	1	5		30	30	30	60	PI/AO		
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		1	1	1	5	30	30			60	Fluoroquench		
262	Yes	Yes	Yes	Yes	Yes	Life Technologies	Yes		1/2	1	1	5		30	60	90		Eosin		
268	Yes	Yes	Yes	Yes	Yes	Gentrack	Yes		1/10	3	27	1	5		30	30	60	Fluoroquench		
284	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M/L	1	19	1	5	35	35	40	40	60	EB/AO		
292	Yes	Yes	Yes	Yes	Yes	One Lambda	Yes	0.015M	1	1	1	3	15	15	30	30	60	Fluoroquench		
293	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	0.05M	50	450			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	Fluoroquench		
297	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.001Mol/ml	1	1	1	6	30	30	30	30	60	Fluoroquench		
311	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	1	1	5	30	30	30	30	60	Fluoroquench		
315	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	1%	4	76	1	5	15	15	30	30	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