

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

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A05+2A06/2016

DESPATCHED ON 07TH JUNE 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	Pre Comp T-cell	Pre Comp B-cell	Post Comp T-cell				Post Comp B-cell	
9		Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	5	95	1	5	30	30	30	30	30	Fluoroquench			
11		Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	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	60	EB/AO			
14		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	Cedarlane	Yes											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	9	81	1	5	30	30	45	45	60	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	1	1	1	5	10	10	45	60	90	EB/AO			
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		0.1	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			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	Cedarlane	Yes		1	1	1	5	0	0	60	60	60	Fluoroquench			
54		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			
101	Yes	Yes	Yes	Yes	Yes	BAG	Yes	0.05M					45	45				Fluoroquench			
112		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			60	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			
117	Yes	Yes	Yes	Yes	Yes	Life Technology	Yes		3, 2, 1	1	5				60		120	Eosin, Fluoroquench	AMOS modification	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		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	Fluoroquench		Sera treatment with DTT prior to CDC incubation: 2µl DTT 0.1M + 38µl serum - incubation 30mn 37°c	
128		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					5			60		60	EB/AO			
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	5	45	1	5	20		30		60	Eosin	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								30	45	45	EB/AO			
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.005M	1	19		5	30	30	30	30	60	Fluoroquench			
149	Yes	Yes	Yes	Yes	Yes	Commerical	Yes		0.5	0.5	1	5			60		60	Eosin			
154	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes								30	30	60	Eosin			
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5			60	60	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	Fluoroquench			
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5			30	30	45	Fluoroquench			
186	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes	5mM	1	1	1	6	30	30	30	30	60	Fluoroquench			
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.00169g/ml	1	1	2	5			60	60	30	EB/AO			
190	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1mg/ml	1	19	1	5	30	30	30	30	60	Fluoroquench			
193	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	0.05	0.95	1	5	30	30	30	30	60	Stain Fix, Hb+PI			
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	1, 2	1, 2	1	5	15	15	30	30	45	Fluoroquench			
197																					
204	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/20	1	1	5	15	15	45	45	90	Fluoroquench			
206	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.5	0.5, 1	0.5	3.3	35	35	30	30	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	Biorad	Yes	1/10	10	90	1	5			30	30	60	PI/AO			
216	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.5M	0.05	0.95	1	6			45	45	60	EB/AO			
227	Yes	Yes	Yes	Yes	Yes	ImmuCOR	Yes	1/9	0.3	2.7	1	5			30	30	60	Fluoroquench	15m incubation of serum with DTT at 37°c before cell/serum deposite		
229	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes								30	30	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			
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								30	30	60	PI/AO			
260	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes		1	1	1	5	30	30	30	30	60	Fluoroquench			
262	Yes	Yes	Yes	Yes	Yes	Life Technologies	Yes	1/2	1	1	1	5			30	60	60	Eosin			
268	Yes	Yes	Yes	Yes	Yes	Gentrack	Yes	1/10				5	30	30	60	60	30	Fluoroquench	AHG on T lymphocytes	T-cells tested with and without AHG	
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		50	450	2400	5	60	60	60	60	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	ID-Bio	Yes	0.01M	0.05	1	1	6	30	30	30	30	60	EB/AO			
311	Yes	Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	95	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		7			30	30	60	Fluoroquench			

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