

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

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A03+2A04/2016

DESPATCHED ON 05TH APRIL 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	Post Comp T-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	60	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					5			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	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	0.01M	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	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	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	BAG	Yes	0.05M					45	45				Fluoroquench		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	One Lambda	Yes	0.05M	0.1	1	1	5	30	30	60	30	60	Fluoroquench			
116		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			3, 2, 1	1	5	60	60	120	120	30	Eosin, Fluoroquench			
119	Yes	Yes	Yes	Yes	Yes	Biorad	Yes		1	1	1	5	30	30	30	60	60	Eosin			
120		Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	6	30	30	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	Eosin			
133		Yes	Yes	Yes	Yes	Innotrain	Yes	0.01M	1	1	1	5	60	60	60	60	60	EB/AO		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											EB/AO			
139	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	5	45	1	5	20	30	30	60	60	Eosin	Washes		
143	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	1:10	1	1	2	5	45	30	30	60	60	Eosin			
145	Yes	Yes	Yes	Yes	Yes	Invitrogen	Yes		1	19			30	30	45	45	45	EB/AO			
147	Yes	Yes	Yes	Yes	Yes	In-house	Yes	0.005M	0.5	0.5	1	5	30	30	30	30	60	Fluoroquench			
149	Yes	Yes	Yes	Yes	Yes	Commercial	Yes											Eosin			
154		Yes	Yes	Yes	Yes	Cedarlane	Yes											Eosin			
157	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.005M	1	1	1	5	60	60	120	120	60	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	30	35	45	75	75	Fluoroquench			
181	Yes	Yes	Yes	Yes	Yes	Cedarlane	Yes	0.05M	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	Fluoroquench			
189	Yes	Yes	Yes	Yes	Yes	Innotrain	Yes	0.016g/10mL	2	2	2	5	37	37	60	60	40	EB/AO			
193	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	Fluoroquench			
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	1, 2	1, 2	1	5	15	15	30	30	60	Fluoroquench			
197		Yes	Yes	Yes	Yes	Cedarlane	Yes		1/20	1	1	5	15	15	45	45	90	Fluoroquench			
204		Yes	Yes	Yes	Yes	Cedarlane	Yes	0.01M	0.5	0.5, 1	0.5	3.3	35	35	30	30	45	45	Fluoroquench		
206	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	
209	Yes	Yes	Yes	Yes	Yes		Yes														
212	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	1/10	10	90	1	5	30	30	60	60	60	PI/AO			
216		Yes	Yes	Yes	Yes	Innotrain	Yes											EB/AO			
227		Yes	Yes	Yes	Yes	Immucor	Yes	0.5M	0.05	0.95	1	6	30	30	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	30	60	60	Fluoroquench			
232	Yes	Yes	Yes	Yes	Yes	Biotest	Yes			0.5	0.5	2.5	45	60	60	60	60	EB/AO			
235	Yes	Yes	Yes	Yes	Yes	Biorad	Yes	0.001M	1	1	1	5	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		
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	60	60	Fluoroquench			
259	Yes	Yes	Yes	Yes	Yes	Biorad	Yes		1	1	1	5	30	30	30	30	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	5	30	30	60	60	90	Eosin			
269		Yes	Yes	Yes	Yes	Gentrack	Yes		3	27	1	5	30	30	60	60	60	Fluoroquench		CDC on T-cells with and without AHG. CDC on B-cells without AHG	
284		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	One Lambda	Yes	0.015M	1	1	1	3	15	15	30	30	60	Fluoroquench		T-cells tested with and without AHG	
293		Yes	Yes	Yes	Yes	Invitrogen	Yes		50	450	2400	5	60	60	60	60	120	120	Eosin		
294		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	60	EB/AO		
311		Yes	Yes	Yes	Yes	Servbio	Yes	0.1M	5	95	1	5	30	30	30	30	60	60	Fluoroquench		
315		Yes	Yes	Yes	Yes	Servbio	Yes	1%	4	76	1	5	15	15	30	30	50	50	Fluoroquench		
321		Yes	Yes	Yes	Yes	Cedarlane	Yes	0.1M	2	38	7	7	30	30	60	60	60	Fluoroquench			

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