

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

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

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	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	1	1	5	30	30	30	30	Fluoroquen						
11	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	1	1	5	30	30	45	45	90	90	Fluoroquen				
12	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.01M	1	1	5	30	30	30	30	60	90	EB/AO				
14	Yes	Yes	Yes	Yes	Cedarlane	Yes		10%	0.1	0.9	1	5	10	10	45	45	75	75	Fluoroquen			
15	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.02M	1	2	1	5	30	30	60	60	60	60	Fluoroquen			
20	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.01M	1	1	1	5	30	30	30	30	60	60	EB/AO			
23	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	1	1	5	40	40	40	40	40	40	EB/AO			
24	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.005M	4.36	1	1	5	30	30	45	45	90	90	Fluoroquen			
25	Yes	Yes	Yes	Yes	Cedarlane	Yes		1-10	9	81	1	5	30	30	45	45	60	60	Fluoroquen			
28	Yes	Yes	Yes	Yes	Cedarlane	Yes	Yes	0.01M	1	1	1	5	30	30	30	30	45	45	EB/AO			
34	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.01M	1	1	1	5	30	30	30	30	90	90	EB/AO			
35	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	1	1	1	5	10	10	45	45	90	90	EB/AO			
38	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.01M	1	1	1	5	60	60	60	60	60	60	EB/AO			
39	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1	1	1	1	5	10	10	45	45	90	90	Fluoroquen			
41	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	1	1	1	5	30	30	45	45	45	45	EB/AO			
42	Yes	Yes	Yes	Yes	TSC Biosciences	Yes	Yes	12mg/ml	1	1	1	5	60	60	120	60	60	60	Fluoroquen			
45	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.005M	1	1	1	5	30	30	60	30	90	75	EB/AO			
51	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	1	1	5	0	0	60	60	60	60	Fluoroquen			
54	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	1	1	5	60	60	60	60	60	60	PI/AO			
58	Yes	Yes	Yes	Yes	Cedarlane	Yes			1	2	1	5	45	45	60	60	90	90	Fluoroquen			
62	Yes	Yes	Yes	Yes	Cedarlane	Yes													EB/AO	One hour incubation with cells, two hours with complement		
101	Yes	Yes	Yes	Yes	BAG	Yes		0.05M				45	45						Fluoroquen			
112	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		60	30	60	30	60	Fluoroquen		
116	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.05		1-5	1	2-4	30	30	20	30	30	30	30	EB/AO	AMOS modification	DTT treatment of the sera is done in a separate tube #1 DTT and 36µl sera. Cells extracted with Rosettesep
117	Yes	Yes	Yes	Yes	Life Technology	Yes				3, 2, 1	1	5			60	120			Eosin, Fluoroquen			
119	Yes	Yes	Yes	Yes	Biorad	Yes		0.005M	1	1	1	5	30		30	60			Eosin			
120	Yes	Yes	Yes	Yes	Cedarlane	Yes						6	30	30	30	60			Fluoroquen		Sera treatment with DTT prior to CDC incubation: 2µl DTT 0.1M + 38µl serum - incubation 30min 37°C	
128	Yes	Yes	Yes	Yes	One Lambda	Yes						1	5		45	90			Eosin			
133	Yes	Yes	Yes	Yes	Innotrain	Yes	Yes	0.01M	1	1	1	5	90	90	30	30	60	60	60	Fluoroquen		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	In-house	Yes						1	5		60	60			EB/AO			
139	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.05M	5	45	1	5	20		30	60			Fluoroquen	Washes		
143	Yes	Yes	Yes	Yes	Cedarlane	Yes		1:10	1	1	2	5	45		30	60			Eosin			
145	Yes	Yes	Yes	Yes	Invitrogen	Yes									45	45	45	45	45	EB/AO		
147	Yes	Yes	Yes	Yes	In-house	Yes		0.005M	1	19		5	30	30	30	30	60	60	60	Fluoroquen		
149	Yes	Yes	Yes	Yes	Commerical	Yes			0.5	0.5	1	5			60	60			Eosin			
154	Yes	Yes	Yes	Yes	Cedarlane	Yes	Yes								30	30	60	60	60	Eosin		
157	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.005M	1	1	1	5			60	60	120	120	120	Fluoroquen		
159	Yes	Yes	Yes	Yes	In-House	Yes		0.05M	10	90	1	5	45	45	30	30	60	60	60	Fluoroquen	AHG incubation during 3 minutes	
162	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.01M	10	90	1	5	45	45	60	60	60	60	60	Fluoroquen		
163	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	1	1	1	5	30	30	30	30	60	60	60	Fluoroquen		
169	Yes	Yes	Yes	Yes	One Lambda	Yes		50mM	1	1	1	5			35	45	75	75	75	Fluoroquen		
181	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.005M	1	1	1	5			30	30	45	45	45	Fluoroquen		
186	Yes	Yes	Yes	Yes	Invitrogen	Yes		5mM	1	19	1	6	30	30	30	30	60	60	60	Fluoroquen		
189	Yes	Yes	Yes	Yes	Innotrain	Yes		0.0016g/ml	1	1	2	5			60	60	30	30	60	EB/AO		
190	Yes	Yes	Yes	Yes	Servbio	Yes		0.1mg/l	1	19	1	5	30	30	30	30	60	60	60	Fluoroquen		
193	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	0.05	0.95	1	5	30	30	30	30	60	60	60	Stain Fix		
194	Yes	Yes	Yes	Yes	In-House	Yes		5%	1	1	1	6	15	15	30	30	45	45	45	Fluoroquen		
195	Yes	Yes	Yes	Yes	Invitrogen	Yes		0.1M	1, 2	1, 2	1	5	15	15	30	30	45	45	45	Fluoroquen		
197																						
204	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/20	1	1	1	5	15	15	45	45	90	90	90	Fluoroquen		
206	Yes	Yes	Yes	Yes	Cedarlane	Yes	Yes	0.01M	0.5	0.5	0.5	3.3			30	30	45	45	45	Fluoroquen		
209	Yes	Yes	Yes	Yes	One Lambda, Ingen	Yes		0.0015M	1	2	1	5	10	10	30	30	60	60	60	Fluoroquen		CDC with AGH on T-lymphocytes during 2 minutes
212																						
216	Yes	Yes	Yes	Yes	Innotrain	Yes		1/10	10	90	1	5			30	30	60	60	60	PI/AO		
223	Yes	Yes	Yes	Yes	Innotrain	Yes									45	45	60	60	60	EB/AO		
227	Yes	Yes	Yes	Yes	Immucor	Yes		0.5M	0.05	0.95	1	6			30	30	60	60	60	Fluoroquen	15m incubation of serum with DTT at 37°C before cell/serum deposit	
229	Yes	Yes	Yes	Yes	Cedarlane	Yes		1/9	0.3	2.7	1	5			30	30	60	60	60	Fluoroquen		
232	Yes	Yes	Yes	Yes	Biotest	Yes				0.5	0.5	2.5			45	60			60	EB/AO		
235	Yes	Yes	Yes	Yes	Biorad	Yes		0.001M	1	1	1	5	30		30	60			60	Eosin		
238	Yes	Yes	Yes	Yes	Invitrogen	Yes		0.1M	0.05	0.95	1	5			45	45	90	90	90	Fluoroquen	Volume serum is 1µl/cells 1µl. But sera are treated with DTT before 1µl DTT + 19µl serum	
245	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	Innotrain	Yes			0.1	1	1	5	30	30			60	60	60	Fluoroquen		
259	Yes	Yes	Yes	Yes	Biorad	Yes									30	30	60	60	60	PI/AO		
260	Yes	Yes	Yes	Yes	Innotrain	Yes			1	1	1	5	30	30	30	30	60	60	60	Fluoroquen		
262	Yes	Yes	Yes	Yes	Life Technologies	Yes		1/2	1	1	1	5			30	60	60	90	90	Eosin		
268	Yes	Yes	Yes	Yes	Gentrack	Yes		1/10	3	27	1	5			30	30	60	90	90	Fluoroquen	XM LCT with AHG on T-cells	
284	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	One Lambda	Yes		0.015M	1	1	1	3	15	15	30	30	60	60	60	Fluoroquen		
293	Yes	Yes	Yes	Yes	Invitrogen	Yes			50	450	2400	5	60	60	60	60	120	120	120	Eosin		
294	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1Mol	1	19	1	3	15	15	30	30	30	30	30	Fluoroquen		
297	Yes	Yes	Yes	Yes	ID-Bio	Yes		0.01M	5	1	1	6	30	30	30	30	60	60	60	EB/AO		
311	Yes	Yes	Yes	Yes	Servbio	Yes		0.1M	5	1	1	5	30	30	30	30	60	60	60	Fluoroquen		
315	Yes	Yes	Yes	Yes	Servbio	Yes		1%	4	76	1	5	15	15	30	30	50	50	50	Fluoroquen		
321	Yes	Yes	Yes	Yes	Cedarlane	Yes		0.1M	2	38	1	7			30	30	60	60	60	Fluoroquen		

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