

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

PBL/T-CELL AND B-CELL CROSSMATCHING METHOD OF SAMPLE 2A01+2A02/2016

DESPATCHED ON 02ND FEBRUARY 2016

| Lab No. | Cells used | | | Immunobeaded cells used? | | Complement Source | DTT addition | | DTT Concentration | Volumes | | | DTT | | Incubation Times | | Post Comp | Visualisation of dead cells | Modifications to the standard NIH CDCXM method | Comments | | |
|---------|------------|---------|---------|--------------------------|---------|-------------------|--------------|-------|-------------------|---------|---------|------|--------|--------|------------------|--------|-----------|-----------------------------|--|---------------------|---|--|
| | PBL | T-cells | B-cells | T-cells | B-cells | | Sera | Cells | | Sera | Cells | Comp | T-cell | B-cell | T-cell | B-cell | | | | | | |
| 9 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | 5 | 95 | 1 | 5 | 30 | 30 | 30 | 30 | Fluoroquench | | | | |
| 11 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | 1 | 1 | 1 | 5 | 30 | 30 | 45 | 45 | 90 | 90 | Fluoroquench | | |
| 12 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.01M | 1 | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 90 | EB/AO | | |
| 14 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 10% | 0.1 | 0.9 | 1 | 5 | 10 | 10 | 45 | 45 | 75 | 75 | Fluoroquench | | |
| 15 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.02M | 1 | 2 | 1 | 5 | 30 | 30 | 60 | 60 | 60 | 60 | Fluoroquench | | |
| 20 | Yes | 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 | Yes | Cedarlane | Yes | | | | 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 | 90 | Fluoroquench | | |
| 25 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | | 1 | 1 | 5 | 30 | 30 | 45 | 45 | 60 | 60 | Fluoroquench | # - Sera pre-treated with DTT 9µl 0.1M DTT + 81µl sera totalling 0.01M DTT | |
| 28 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | Yes | 0.01M | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 45 | 45 | EB/AO | | |
| 34 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.01M | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 90 | 90 | EB/AO | | |
| 35 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | | 1 | 1 | 5 | 10 | 10 | 45 | 45 | 60 | 60 | EB/AO | | |
| 38 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.01M | | 1 | 1 | 5 | 60 | 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 | 90 | Fluoroquench | | |
| 41 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | | 1 | 1 | 5 | 30 | 30 | 45 | 45 | 45 | 45 | EB/AO | # - 36µl of sera incubated with 2µl of DTT prior to addition to well | |
| 42 | Yes | Yes | Yes | Yes | Yes | TSC Biosciences | Yes | Yes | 12mg/ml | | 1 | 1 | 5 | 60 | 60 | 120 | 60 | 120 | 60 | Fluoroquench | | |
| 45 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.005M | | 1 | 1 | 5 | 30 | 30 | 60 | 30 | 90 | 75 | EB/AO | | |
| 51 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | | | 1 | 1 | 5 | 0 | 0 | 60 | 60 | 60 | 60 | Fluoroquench | | |
| 54 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | | | 1 | 1 | 5 | 60 | 60 | 60 | 60 | 60 | 60 | PI/AO | | |
| 58 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | | | 1 | 2 | 5 | 45 | 45 | 60 | 60 | 90 | 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 | 60 | 30 | 60 | Fluoroquench | | |
| 116 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.05 | | 1-5 | 1 | 2-4 | 30 | 30 | 20 | 20 | 30 | 30 | EB/AO | AMOS modification | |
| 117 | Yes | Yes | Yes | Yes | Yes | Life Technology | Yes | | | | 3, 2, 1 | 1 | 5 | | | 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 | 6 | 30 | 30 | 30 | 60 | 60 | 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 | Yes | One Lambda | Yes | | | | 1 | 1 | 5 | | | 45 | | 90 | | Eosin | | |
| 133 | Yes | Yes | Yes | Yes | Yes | Inotrain | Yes | | 0.01M | | 1 | 1 | 5 | 90 | 90 | 30 | 30 | 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 | Inotrain | Yes | | | | 1 | 1 | 5 | | | 35 | | 75 | | EB/AO | | |
| 139 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.05M | | 5 | 1 | 5 | 20 | 20 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 142 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 1:10 | | 1 | 1 | 2 | 5 | 45 | | 30 | | 60 | | Eosin | |
| 143 | Yes | Yes | Yes | Yes | Yes | Invitrogen | Yes | | | | 2 | 2 | 5 | 37 | 37 | 60 | 60 | 40 | 40 | EB/AO | | |
| 147 | Yes | Yes | Yes | Yes | Yes | In-house | Yes | | 0.005M | | 1 | 19 | | 30 | 30 | 30 | 30 | 60 | 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 | 60 | 60 | Eosin | | |
| 157 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | Yes | 0.005M | | 1 | 1 | 5 | 60 | 60 | 120 | 120 | 120 | 120 | Fluoroquench | AHG incubation during 3 minutes | |
| 159 | Yes | Yes | Yes | Yes | Yes | In-House | Yes | | 10% | 1 | 1 | 1 | 5 | 45 | 45 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 162 | Yes | Yes | Yes | Yes | Yes | One Lambda | Yes | | 0.01M | 10 | 90 | 1 | 5 | | | 60 | 60 | 60 | 60 | Fluoroquench | | |
| 163 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 169 | Yes | Yes | Yes | Yes | Yes | One Lambda | Yes | | 5mM | | 1 | 1 | 5 | 30 | 30 | 35 | 35 | 75 | 75 | Fluoroquench | | |
| 181 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.05M | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 45 | 45 | Fluoroquench | | |
| 186 | Yes | Yes | Yes | Yes | Yes | Invitrogen | Yes | | 5mM | | 1 | 19 | 6 | 30 | 30 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 189 | Yes | Yes | Yes | Yes | Yes | Inotrain | Yes | | 0.0169/10mL | | 2 | 2 | 5 | 37 | 37 | 60 | 60 | 40 | 40 | EB/AO | | |
| 190 | Yes | Yes | Yes | Yes | Yes | Servbio | Yes | | 0.1mg/l | | 1 | 19 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 193 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1M | 0.05 | 0.95 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Hb/PI | | |
| 194 | Yes | Yes | Yes | Yes | Yes | In-House | Yes | | 5% | | 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 | 45 | 45 | Fluoroquench | | |
| 197 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | | 1/20 | 1 | 1 | 5 | 15 | 15 | 45 | 45 | 90 | 90 | Fluoroquench | | |
| 204 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | Yes | 0.01M | 0.5 | 0.5 | 1 | 3.3 | 35 | 35 | 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 | 60 | Fluoroquench | CDC with AGH on T-lymphocytes during 2 minutes |
| 212 | Yes | Yes | Yes | Yes | Yes | Biorad | Yes | | 1/10 | 10 | 90 | | 5 | | | 30 | | 60 | | PI/AO | | |
| 216 | Yes | Yes | Yes | Yes | Yes | Inotrain | Yes | | | | | | | | | 45 | | 45 | | EB/AO | | |
| 223 | Yes | Yes | Yes | Yes | Yes | Inmucor | Yes | | 0.5M | 0.05 | 0.95 | 1 | 6 | | | 30 | | 30 | | 60 | 60 | |
| 227 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 1/9 | 0.3 | 2.7 | 1 | 5 | | | 30 | | 30 | | 60 | 60 | |
| 229 | Yes | Yes | Yes | Yes | Yes | Biotest | Yes | | | | 0.5 | 0.5 | 2.5 | | | 45 | | 60 | | EB/AO | | |
| 232 | Yes | Yes | Yes | Yes | Yes | Biorad | Yes | | 0.001M | | 1 | 1 | 5 | 30 | | 30 | | 60 | | Eosin | | |
| 235 | Yes | Yes | Yes | Yes | Yes | Invitrogen | Yes | | 1/20 | 2 | 38 | | 5 | 30 | 30 | 30 | 45 | 60 | 90 | Fluoroquench | | |
| 238 | Yes | Yes | Yes | Yes | Yes | Biorad | Yes | | | | 1 | 1 | 5 | | | 30 | | 30 | | 60 | 60 | |
| 243 | Yes | Yes | Yes | Yes | Yes | BAG | Yes | | 0.1M% | 5 | 95 | 1, 2 | 5 | 30 | 30 | 30 | 30 | 60 | 45 | EB/AO | | |
| 245 | Yes | Yes | Yes | Yes | Yes | Commercial | Yes | | | 0.1 | 1 | 1 | 5 | 30 | 30 | | | 60 | 60 | Fluoroquench | | |
| 252 | Yes | Yes | Yes | Yes | Yes | Biorad | Yes | | | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | PI/AO | | |
| 259 | Yes | Yes | Yes | Yes | Yes | Inotrain | Yes | | 0.01% | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 260 | Yes | Yes | Yes | Yes | Yes | In-House | Yes | | 1/2 | | 1 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Eosin | | |
| 262 | Yes | Yes | Yes | Yes | Yes | Life Technologies | Yes | | | | 1 | 1 | 5 | 30 | 30 | 60 | 60 | 90 | 90 | Fluoroquench | | |
| 268 | Yes | Yes | Yes | Yes | Yes | Gentrack | Yes | | 0.05M | 3 | 27 | 1 | 5 | 45 | 45 | 30 | 30 | 60 | 90 | Fluoroquench | CDC with AGH on T-lymphocytes | |
| 284 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1ML | 1 | 19 | 1 | 5 | 35 | 35 | 40 | 40 | 60 | 60 | EB/AO | T-cells tested with and without AHG | |
| 292 | Yes | Yes | Yes | Yes | Yes | One Lambda | Yes | | 0.015M | | 1 | 1 | 3 | 15 | 15 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 293 | Yes | Yes | Yes | Yes | Yes | Invitrogen | Yes | | | 50 | 450 | 2400 | 5 | 60 | 60 | 60 | 60 | 120 | 120 | Eosin | | |
| 294 | Yes | Yes | Yes | Yes | Yes | Cedarlane | Yes | | 0.1Mol | | 1 | 19 | 3 | 15 | 15 | 30 | 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 | Yes | Servbio | Yes | | 0.1M | 5 | 95 | 1 | 5 | 30 | 30 | 30 | 30 | 60 | 60 | Fluoroquench | | |
| 315 | Yes | Yes | Yes | Yes | Yes | Servbio | Yes | | 1% | 4 | 76 | 1 | 5 | 15 | 15 | 30 | 30 | 50 | 50 | Fluoroquench | | |

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