Test Facility : IIVS
Chemical Code : N/A
2nd Chem. Code*: NRU

Study Number.: R&D - NR Stain Time Course in NHK
96-Well Plate ID : 1
Experiment ID : RD9602NK

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank											Blank
C	Blank											Blank
D	Blank	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min	Blank
E	Blank											Blank
F	Blank											Blank
G	Blank											Blank
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.057	0.053	0.059	0.058	0.054	0.055	0.053	0.052	0.051	0.048	0.049	0.035
B	0.068	1.501	1.564	1.311	1.327	0.998	1.052	0.671	0.649	0.438	0.474	0.037
C	0.057	1.549	1.482	1.376	1.372	1.082	1.076	0.714	0.697	0.494	0.474	0.034
D	0.058	1.540	1.503	1.415	1.422	1.026	0.995	0.724	0.698	0.482	0.474	0.036
E	0.057	1.553	1.532	1.388	1.453	1.060	1.010	0.675	0.634	0.459	0.462	0.034
F	0.057	1.632	1.600	1.396	1.380	1.066	1.074	0.656	0.628	0.470	0.429	0.033
G	0.054	1.462	1.514	1.357	1.439	1.069	1.010	0.708	0.606	0.474	0.437	0.035
H	0.057	0.054	0.053	0.052	0.051	0.055	0.051	0.049	0.047	0.050	0.046	0.034

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

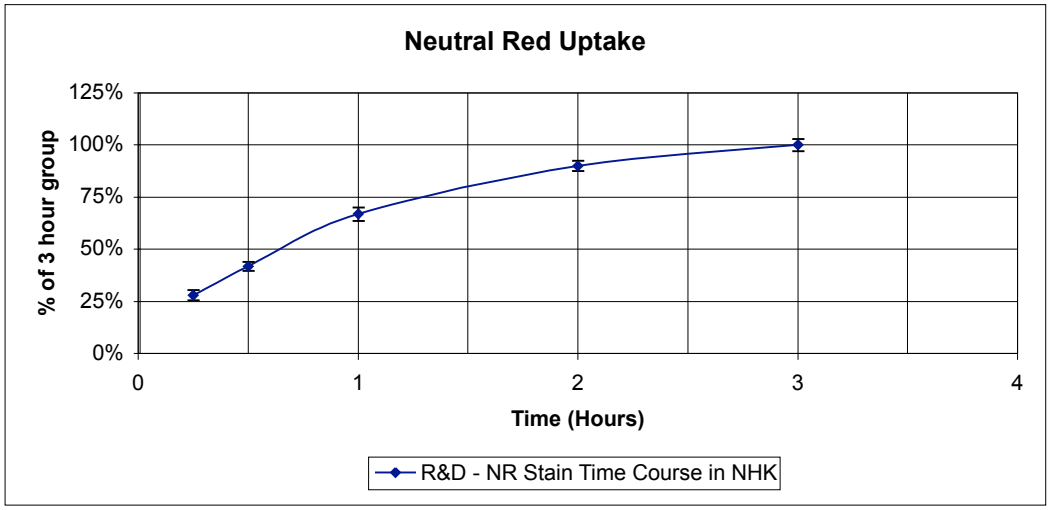
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.007	0.003	0.009	0.008	0.004	0.005	0.003	0.002	0.001	-0.002	-0.001	-0.015
B	0.018	1.451	1.514	1.261	1.277	0.948	1.002	0.621	0.599	0.388	0.424	-0.013
C	0.007	1.499	1.432	1.326	1.322	1.032	1.026	0.664	0.647	0.444	0.424	-0.016
D	0.008	1.490	1.453	1.365	1.372	0.976	0.945	0.674	0.648	0.432	0.424	-0.014
E	0.007	1.503	1.482	1.338	1.403	1.010	0.960	0.625	0.584	0.409	0.412	-0.016
F	0.007	1.582	1.550	1.346	1.330	1.016	1.024	0.606	0.578	0.420	0.379	-0.017
G	0.004	1.412	1.464	1.307	1.389	1.019	0.960	0.658	0.556	0.424	0.387	-0.015
H	0.007	0.000	0.003	0.002	0.001	0.005	0.001	-0.001	-0.003	0.000	-0.004	-0.016

Mean Blank =	0.050

RELATIVE VIABILITY (% OF VEHICLE CONTROL)

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B		97.6%	101.9%	84.9%	85.9%	63.8%	67.4%	41.8%	40.3%	26.1%	28.6%	
C		100.9%	96.4%	89.2%	89.0%	69.5%	69.1%	44.7%	43.6%	29.9%	28.6%	
D		100.3%	97.8%	91.9%	92.3%	65.7%	63.6%	45.4%	43.6%	29.1%	28.6%	
E		101.1%	99.7%	90.0%	94.4%	68.0%	64.6%	42.1%	39.3%	27.5%	27.7%	
F		106.5%	104.3%	90.6%	89.5%	68.4%	68.9%	40.8%	38.9%	28.3%	25.5%	
G		95.0%	98.5%	88.0%	93.5%	68.6%	64.6%	44.3%	37.4%	28.6%	26.1%	
H												

	3 hr	3 hr	2 hr	2 hr	1 hr	1 hr	30 min	30 min	15 min	15 min
Conc. (µg/mL) :										
Mean Corr. OD :	1.490	1.483	1.324	1.349	1.001	0.987	0.642	0.602	0.420	0.409
SD :	0.057	0.043	0.036	0.048	0.032	0.036	0.028	0.038	0.019	0.020
Mean 3 hour :	1.486									
Mean Blank :	0.050									
% of 3 hour:	100.2%	99.8%	89.1%	90.8%	67.3%	66.4%	43.2%	40.5%	28.3%	27.5%
SD :	3.8%	2.9%	2.4%	3.2%	2.1%	2.4%	1.9%	2.5%	1.3%	1.4%
% CV :	3.83%	2.91%	2.75%	3.53%	3.17%	3.61%	4.29%	6.28%	4.62%	4.97%
hours			3	2	1	0.50	0.25			
% of 3 hour:			100.0%	89.9%	66.8%	41.9%	27.9%			


Neutral Red Uptake
125%
100%
75%
50%
25%
0%
% of 3 hour group
0
1
2
3
4
Time (Hours)
R&D - NR Stain Time Course in NHK

Neutral Red Stain Prepared in KGM - TEST OF NR PREP 1 DAY PRIOR TO USE
Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

	1	2	3	4	5	6	7	8	9	10	11
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank
B	Blank										
C	Blank	50 ug/ml			50 ug/ml				33 ug/ml		
D	Blank	Prepared and filtered			Filtered before use				Filtered before use		
E	Blank	in evening before use									
F	Blank	Filtered before use									
G	Blank										
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11
A	0.062	0.061	0.063	0.064	0.063	0.062	0.060	0.060	0.052	0.053	0.051
B	0.055	1.306	1.545	1.530	1.514	1.403	1.421	1.297	1.249	1.136	1.134
C	0.060	1.530	1.520	1.554	1.471	1.536	1.416	1.415	1.308	1.160	1.189
D	0.062	1.454	1.527	1.513	1.511	1.472	1.491	1.438	1.217	1.192	1.173
E	0.067	1.423	1.433	1.505	1.577	1.469	1.448	1.474	1.199	1.249	1.158
F	0.057	1.423	1.591	1.577	1.577	1.403	1.431	1.347	1.250	1.235	1.102
G	0.065	1.430	1.468	1.393	1.319	1.432	1.304	1.416	1.243	1.117	1.110
H	0.064	0.059	0.060	0.064	0.064	0.065	0.061	0.064	0.060	0.055	0.060

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11
A	0.012	0.011	0.013	0.014	0.013	0.012	0.010	0.010	0.002	0.003	0.001
B	0.005	1.256	1.495	1.480	1.464	1.353	1.371	1.247	1.199	1.086	1.084
C	0.010	1.480	1.470	1.504	1.421	1.486	1.366	1.365	1.258	1.110	1.139
D	0.012	1.404	1.477	1.463	1.461	1.422	1.441	1.388	1.167	1.142	1.123
E	0.017	1.373	1.383	1.455	1.527	1.419	1.398	1.424	1.149	1.199	1.108
F	0.007	1.373	1.541	1.527	1.527	1.353	1.381	1.297	1.200	1.185	1.052
G	0.015	1.380	1.418	1.343	1.269	1.382	1.254	1.366	1.193	1.067	1.060
H	0.014	0.000	0.010	0.014	0.014	0.015	0.011	0.014	0.010	0.005	0.010

Mean Blank = 0.055 (Only the 14 wells from the 33 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :		50.0			50.0				33.0	
Mean Corr. OD :	1.378	1.464	1.462	1.445	1.403	1.369	1.348	1.195	1.132	1.095
SD :	0.072	0.056	0.064	0.096	0.051	0.062	0.064	0.037	0.053	0.035
Group mean corr OD:		1.435			1.391				1.141	

Note: No crystal formation was observed in the KGM/NR prepared 1 day prior.
No ppt or crystalization observed in the wells during the NR loading of cells.

Neutral Red Stain Prepared in KGM/Filtered immediately before use
Tested in 90-100% Confluent NHK Cultures

96-WELL PLATE MAP

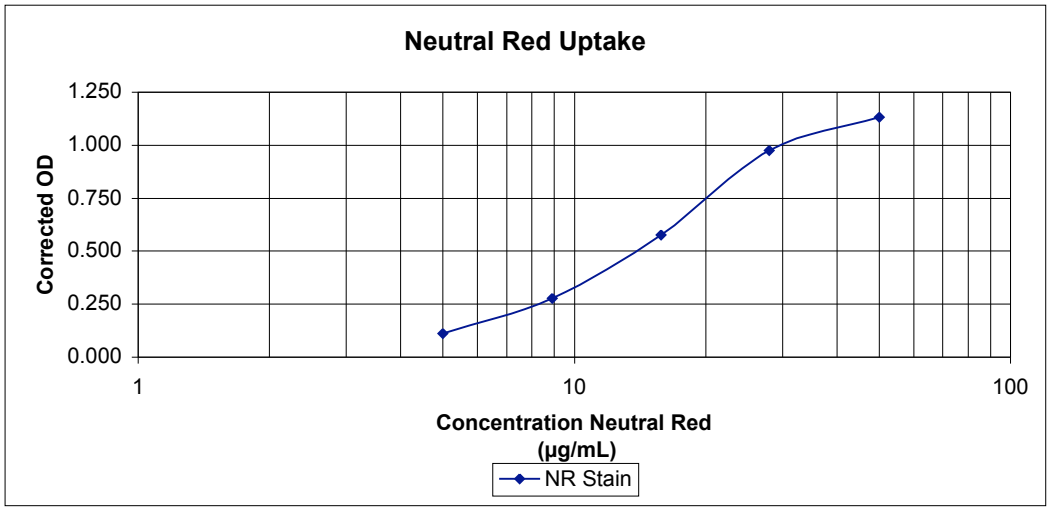
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	
B	Blank	50 ug/ml	50 ug/ml	28 ug/ml	28 ug/ml	16 ug/ml	16 ug/ml	9 ug/ml	9 ug/ml	5 ug/ml	5 ug/ml	
C	Blank											
D	Blank											empty
E	Blank											
F	Blank											
G	Blank											
H	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	Blank	

RAW ABSORBANCE DATA (OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.067	0.064	0.066	0.049	0.049	0.040	0.040	0.038	0.038	0.036	0.037	0.035
B	0.048	1.255	1.119	1.103	1.054	0.623	0.605	0.325	0.334	0.156	0.150	0.034
C	0.050	1.035	1.004	1.020	0.956	0.624	0.601	0.345	0.312	0.151	0.154	0.034
D	0.047	1.131	1.352	1.094	1.078	0.643	0.635	0.331	0.314	0.157	0.147	0.035
E	0.047	1.117	1.227	0.923	0.893	0.595	0.618	0.323	0.302	0.155	0.150	0.035
F	0.046	1.245	1.129	0.976	0.988	0.607	0.617	0.308	0.313	0.156	0.156	0.035
G	0.047	1.136	1.282	1.061	0.995	0.624	0.582	0.283	0.282	0.131	0.127	0.037
H	0.063	0.056	0.060	0.061	0.048	0.042	0.042	0.038	0.039	0.040	0.038	0.036

CORRECTED ABSORBANCE (Sample OD_{550} - Mean Blank OD_{550})

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.017	0.014	0.016	-0.001	-0.001	-0.010	-0.010	-0.012	-0.012	-0.014	-0.013	-0.015
B	-0.002	1.205	1.069	1.053	1.004	0.573	0.555	0.275	0.284	0.106	0.100	-0.016
C	0.000	0.985	0.954	0.970	0.906	0.574	0.551	0.295	0.262	0.101	0.104	-0.016
D	-0.003	1.081	1.302	1.044	1.028	0.593	0.585	0.281	0.264	0.107	0.097	-0.015
E	-0.003	1.067	1.177	0.873	0.843	0.545	0.568	0.273	0.252	0.105	0.100	-0.015
F	-0.004	1.195	1.079	0.926	0.938	0.557	0.567	0.258	0.263	0.106	0.106	-0.015
G	-0.003	1.086	1.232	1.011	0.945	0.574	0.532	0.233	0.232	0.081	0.077	-0.013
H	0.013	0.000	0.010	0.011	-0.002	-0.008	-0.008	-0.012	-0.011	-0.010	-0.012	-0.014

Mean Blank = 0.038 (Only the 4 wells from the 5.0 ug/ml group)

	Neutral Red Stain Concentration									
Conc. (µg/mL) :	50.0	50.0	28.0	28.0	15.8	15.8	8.9	8.9	5.0	5.0
Mean Corr. OD :	1.104	1.136	0.980	0.944	0.570	0.560	0.270	0.260	0.101	0.098
SD :	0.083	0.126	0.070	0.067	0.017	0.018	0.021	0.017	0.010	0.010
Group mean corr OD:	1.120		0.962		0.565		0.265		0.100	
	graph	x	50.0	28.0	15.8	8.9	5.0			
		y	1.120	0.962	0.565	0.265	0.100