






Supplementary data to:

Original article:

***IN VITRO* SCREENING OF TOPICAL FORMULATION EXCIPIENTS
FOR EPITHELIAL TOXICITY IN CANCEROUS AND
NON-CANCEROUS CELL LINES**

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Supplement 1

Raw data shows optical density (OD) measurement of dissolved purple formazan in DMSO observed from MTT assay (**S1-Tables 1-4**) and optical density (OD) measurement of dissolved crystal violet dye in DMSO from crystal violet (CV) assay (**S1-Tables 5-8**) after 24 h treatment. Different concentrations of four tested excipients Labrasol®, Labrafil®, Transcutol®, and Labrafac® were applied on three cancer cell lines (WM164, WM1366, and D24) and two normal cells (HaCaT and primary fibroblasts).

Raw data tables concerning:

Figure 1: Representative dose-response curves from 24-h exposure of the tested cell lines to the tested excipients Labrasol®, Labrafil®, Transcutol®, and Labrafac®

Figure 2: a) Cell viability % obtained from MTT assay and calculated as percentage of untreated control. b) Cytotoxicity of the excipients expressed as IC₅₀ values (µg/ml) on melanoma (WM164, WM1366, and D24) and Normal (HaCaT and FB) cells and measured by MTT assay.

Table 3: IC₅₀ values (µg/ml) of tested excipients and Triton-X-100 as positive control, obtained by MTT, CV assays in the used human cell lines.

Figure 3: Correlation of cell viability values (%) between MTT and CV assays for all surfactants in each cell line.

Figure 4: Cytotoxicity of the excipients expressed as IC₅₀ (µg/ml) on the tested cell lines and measured by MTT and CV assays.

(-) These concentrations have not been tested in some cell lines. Excipient concentrations were selected based on the sensitivity of the tested cell lines.

MTT assay (S1-Tables 1-4): After cell incubation for 24 h, the excipient-containing medium was removed, and 100 µl of MTT in PBS (5 mg/ml) diluted 1:10 in medium was then added in the dark. Plates were further incubated for 2-3 h, after which time the medium was removed and the purple formazan product was dissolved by adding 200 µl of DMSO to each well. Plates were then placed in a shaker for 5 min at room temperature and the absorbance of the resulting solutions was measured at 570 nm using a microplate reader (Multiskan™ FC Microplate Photometer).

S1-Table 1:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast				
Labrasol (µg/mL)																									
0.0	1.806	1.943	1.908	1.852	1.632	1.107	1.102	1.137	1.122	1.125	1.288	1.364	1.145	1.229	1.211	1.107	1.102	1.137	1.122	1.095	0.462	0.458	0.401	0.446	0.462
0.1	1.327	1.478	1.786	1.407	1.323	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.436	0.437	0.436	0.426	0.436
0.2	0.824	0.852	0.922	0.895	0.900	1.000	1.111	1.019	1.082	1.081	1.188	1.209	1.162	1.283	1.085	1.099	1.111	1.019	1.082	1.081	0.366	0.366	0.384	0.375	0.366
0.3	0.187	0.189	0.209	0.194	0.191	0.614	0.597	0.580	0.601	0.592	0.779	0.699	0.795	0.705	0.766	0.614	0.597	0.580	0.601	0.572	0.322	0.318	0.319	0.324	0.322
1	0.114	0.117	0.112	0.121	0.123	0.130	0.144	0.122	0.124	0.123	0.120	0.117	0.121	0.118	0.119	0.130	0.144	0.122	0.124	0.123	0.148	0.152	0.155	0.154	0.148

S1-Table 2:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast				
Labrafil (µg/mL)																									
0.0	1.782	1.732	1.696	1.741	1.697	1.080	1.119	1.015	1.123	1.052	1.447	1.314	1.358	1.362	1.412	2.279	2.280	2.334	2.109	1.976	0.462	0.458	0.401	0.446	0.462
0.1	1.516	1.672	1.569	1.651	1.517	1.095	0.929	1.007	0.999	1.062	1.234	1.174	1.191	1.173	1.185	1.735	1.635	1.698	1.764	1.714	0.439	0.394	0.434	0.448	0.439
0.2	-	-	-	-	-	-	-	-	-	-	1.211	1.207	1.173	1.255	1.219	-	-	-	-	-	-	-	-	-	-
1	1.363	1.487	1.374	1.610	1.289	0.706	0.757	0.742	0.763	0.725	0.676	0.654	0.685	0.698	0.691	1.394	1.443	1.451	1.391	1.485	0.380	0.293	0.382	0.270	0.380
2	0.789	0.899	0.721	0.821	0.790	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.236	0.237	0.233	0.232	0.236
3	-	-	-	-	-	-	-	-	-	-	0.674	0.514	0.644	0.676	0.626	-	-	-	-	-	-	-	-	-	-
4	0.195	0.135	0.120	0.122	0.118	0.134	0.114	0.114	0.114	0.121	1.196	0.998	1.214	1.220	1.111	0.300	0.284	0.252	0.297	0.281	-	-	-	-	-
5	-	-	-	-	-	-	-	-	-	-	0.131	0.131	0.118	0.123	0.122	-	-	-	-	-	0.203	0.200	0.227	0.224	0.203
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.172	0.168	0.171	0.175	0.172

S1-Table 3:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast				
Transcutol (µg/mL)																									
0.0	1.782	1.732	1.696	1.741	1.697	1.080	1.119	1.015	1.123	1.052	1.4467	1.3142	1.3582	1.3615	1.4116	2.279	2.280	2.334	2.109	1.976	0.462	0.458	0.401	0.446	0.436
0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.969	2.035	1.978	1.965	2.165	0.428	0.462	0.443	0.416	0.433
1	1.659	1.551	1.649	1.548	1.683	1.052	1.033	1.033	1.094	1.012	1.3547	1.2811	1.3262	1.3154	1.3082	1.307	1.258	1.294	1.287	1.290	0.415	0.398	0.398	0.446	0.436
2	0.847	0.919	0.896	0.929	0.919	0.859	0.828	0.843	0.827	0.802	1.1967	1.1261	1.1353	1.1206	1.1133	0.913	0.948	0.990	0.859	0.701	0.276	0.261	0.280	0.290	0.306
5	0.196	0.187	0.192	0.195	0.217	0.335	0.364	0.341	0.357	0.317	0.7255	0.6323	0.6996	0.6671	0.7209	-	-	-	-	-	0.285	0.253	0.229	0.251	0.296
10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.143	0.142	0.142	0.141	0.140

S1-Table 4:

Cell line	WM164					WM1366					D24				HaCaT					Fibroblast				
Labrafac (µg/mL)																								
0.0	-	-	-	-	-	-	-	-	-	-	1.2875	1.364	1.1451	1.229	1.037	1.067	1.119	1.086	1.048	0.462	0.458	0.401	0.446	0.436
0.0	2.838	2.913	2.609	1.627	1.632	1.107	1.102	1.137	1.122	1.125	2.569	2.5531	2.5617	-	1.107	1.102	1.137	1.122	1.095	-	-	-	-	-
20	1.374	1.467	1.537	1.341	1.298	1.090	1.074	1.079	1.115	1.084	1.259	1.188	1.123	1.199	1.060	1.074	1.079	1.115	1.084	0.416	0.409	0.411	0.406	0.406
50	-	-	-	-	-	-	-	-	-	-	0.868	0.942	0.835	0.858	-	-	-	-	-	0.434	0.453	0.440	0.455	0.441
100	2.368	2.445	2.435	-	-	-	-	-	-	-	1.640	1.789	1.533	-	-	-	-	-	-	0.573	0.589	0.563	0.457	0.540
200	2.072	2.039	2.639	-	-	1.060	1.074	1.079	1.115	1.318	1.888	1.833	1.979	-	1.060	1.074	1.079	1.115	1.283	0.582	0.683	0.602	0.552	0.511
500	-	-	-	-	-	0.723	0.878	1.001	0.985	1.005	-	-	-	-	0.988	0.717	0.853	1.150	1.157	-	-	-	-	-

S1-Table 4: (Positive Control)

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast				
Triton X-100 (µg/mL)																									
0.0	2.838	2.913	2.609	2.589		0.639	0.632	0.449	0.540	0.602	0.815	0.790	0.781	0.803	0.787	1.630	1.482	1.596	1.523	1.785	0.721	0.689	0.661	0.750	
0.1	-	-	-	-		0.736	0.619	0.675	0.606	0.715	-	-	-	-	-	-	-	-	-	-	0.714	0.658	0.638	0.735	
0.3	0.153	0.156	0.154	0.143		-	-	-	-	-	0.744	0.757	0.767	0.792	0.748	1.427	1.248	1.405	1.364	1.546	0.578	0.567	0.584	0.627	
0.5	0.145	0.165	0.167	0.148		-	-	-	-	-	0.144	0.122	0.147	0.147	0.136	0.332	0.287	0.363	0.396	0.383	0.381	0.399	0.387	0.418	
1	-	-	-	-		0.115	0.122	0.115	0.112	0.126	0.138	0.124	0.144	0.122	0.134	-	-	-	-	-	0.397	0.415	0.364	0.426	
10	0.144	0.108	0.110	0.108		0.087	0.066	0.052	0.088	0.071	-	-	-	-	-	0.155	0.145	0.152	0.151	0.142	-	-	-	-	

Raw data shows optical density (OD) of dissolved crystal violet dye in DMSO at 570 nm (S1-Tables 5-8) after 24 h treatment. Different concentrations of four tested excipients Labrasol®, Labrafil®, Transcutol®, and Labrafac® were applied on three cancer cell lines (WM164, WM1366, and D24) and two normal cells (HaCaT and primary fibroblasts).

Cells were fixed by 4 % (v/v) paraformaldehyde in PBS for 10 minutes. The fixed cells were washed twice with PBS and covered with 250 µl crystal violet working solution. The plates were placed on a bench rocker with medium frequency (20 oscillations per minute) at room temperature for 20 minutes. The plates were emptied and washed under indirect stream of tap water for three times and inverted on filter paper. The plates were taped gently on the paper to remove any remaining liquid and air dried with no lid for minimum of 2 hr at room temperature. After the plates were photographed, blue dye was dissolved in DMSO and the optical density of each well was measured at 570 nm (OD570) with a plate reader (Multiskan™ FC Microplate Photometer).

S1-Table 5:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast		
Labrasol (µg/mL)																							
0.0	3.965	3.896	3.896	3.848	3.797	3.088	3.016	2.994	3.065	3.000	3.752	3.670	3.673	3.668	3.696	3.914	3.876	3.909	3.751	3.780	0.631	0.664	0.530
0.1	3.828	3.891	3.425	-	3.598	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.591	0.579	0.600
0.2	0.325	0.332	0.301	0.347	0.364	1.559	1.559	1.564	1.567	1.595	3.427	3.593	3.361	3.507	3.193	2.867	2.822	2.841	3.024	2.916	0.518	0.529	0.521
0.3	2.079	1.978	2.023	1.982	2.246	2.664	2.666	2.566	2.600	2.594	2.299	2.335	2.242	2.174	2.240	1.744	1.725	1.664	1.759	1.687	0.379	0.377	0.382
1	0.189	0.176	0.175	0.193	0.185	0.167	0.158	0.160	0.159	0.156	0.218	0.217	0.223	0.192	0.194	0.634	0.611	0.671	0.618	0.627	0.272	0.216	0.220

S1-Table 6:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast		
Labrafil (µg/mL)																							
0.0	3.634	3.865	3.658	3.821	3.605	3.218	3.208	3.018	2.998	3.334	3.042	3.014	3.166	-	-	3.921	3.825	3.840	3.959	3.874	0.631	0.664	0.530
0.0	-	-	-	-	-	-	-	-	-	-	3.698	3.763	3.715	3.731	3.672	-	-	-	-	-	-	-	-
0.2	3.940	3.856	3.788	3.749	3.876	3.233	2.852	2.813	3.392	3.778	3.681	3.670	3.721	3.771	4.000	3.956	3.898	3.870	3.810	0.573	0.549	0.648	
1	4.085	4.015	3.952	3.750	4.033	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.657	0.577	0.509
2	2.230	1.842	2.244	1.988	1.952	1.178	1.780	1.672	1.798	1.758	3.444	3.567	3.537	3.424	3.556	3.447	3.565	3.513	3.479	3.188	0.500	0.499	0.521
3	-	-	-	-	-	-	-	-	-	-	1.571	1.566	1.497	-	-	-	-	-	-	-	0.355	0.332	0.313
4	-	-	-	-	-	-	-	-	-	-	1.108	1.093	1.073	-	-	-	-	-	-	-	-	-	-
5	0.285	0.255	0.238	0.276	0.335	0.215	0.214	0.218	0.214	0.216	0.432	0.395	0.428	0.374	0.401	1.061	1.141	1.116	1.042	1.014	-	-	-

S1-Table 7:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast		
Transcutol (µg/mL)																							
0.0	3.634	3.865	3.658	3.821	3.605	3.088	3.016	2.994	3.065	3.000	3.752	3.670	3.673	3.668	3.696	3.921	3.825	3.84	3.9593	3.8743	0.631	0.664	0.530
0.0	-	-	-	-	-	3.218	3.184	3.193	3.196	3.278	3.698	3.763	3.715	3.731	3.672	3.914	3.876	3.909	3.751	3.780	-	-	-
1	3.215	3.296	3.237	3.292	2.981	3.109	3.055	3.040	3.040	3.098	3.598	3.669	3.672	3.566	3.667	3.486	3.641	3.497	3.543	3.260	0.363	0.400	0.384
2	1.821	1.937	1.751	1.755	1.675	2.514	2.527	2.487	2.565	2.614	3.011	3.006	3.003	3.021	3.001	2.410	2.265	2.202	2.298	2.140	0.605	0.566	0.510
5	0.199	0.210	0.216	0.208	0.209	0.995	0.964	0.956	0.984	0.992	1.969	1.874	1.860	1.872	1.950	1.330	1.308	1.326	1.235	1.259	0.363	0.400	0.384

S1-Table 8:

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast		
Labrafac (µg/mL)																							
0.0	3.965	3.896	3.896	3.848	3.797	3.088	3.016	2.994	3.065	3.000	3.7517	3.67	3.6734	3.6676	3.696	3.914	3.876	3.909	3.751	3.780	0.4622	0.4576	0.4785
0.0	-	-	-	-	-	-	-	-	-	-	3.042	3.014	3.166	3.103	3.201	-	-	-	-	-	-	-	-
20	3.956	3.859	3.910	3.843	3.984	2.878	2.996	3.033	3.037	3.004	3.698	3.542	3.822	3.603	3.642	4.000	3.902	3.772	3.850	3.908	0.476	0.458	0.474
100	-	-	-	-	-	2.778	3.327	3.866	3.037	3.004	1.846	1.823	1.881	1.894	1.927	-	-	-	-	-	0.462	0.468	0.499
200	3.494	3.344	3.417	3.311	-	-	-	-	-	-	1.748	1.638	1.692	1.816	1.763	3.989	3.900	3.801	3.860	3.910	-	-	-
500	3.628	3.561	3.616	3.672	-	3.432	2.771	3.287	3.311	3.589	-	-	-	-	-	4.040	4.264	4.287	3.558	3.541	0.467	0.436	0.465

S1-Table 9: (Positive Control)

Cell line	WM164					WM1366					D24					HaCaT					Fibroblast				
Triton X-100 (µg/mL)																									
0.0	3.457	3.312	3.342	3.584	3.725	0.580	0.617	0.622	0.520	0.577	3.042	3.014	3.166	3.158	3.202	1.896	2.153	2.011	1.696	2.210	0.588	0.613	0.501	0.578	0.552
0.1	5.890	5.407	6.228	6.701	-	0.698	0.696	1.060	0.822	0.810	-	-	-	-	-	-	-	-	-	-	0.512	0.545	0.449	0.515	0.483
0.3	0.540	0.684	0.501	-	0.637	-	-	-	-	-	2.777	2.885	3.108	3.115	3.044	1.807	2.007	1.931	1.617	2.083	0.419	0.447	0.356	0.407	0.389
0.5	0.487	0.518	0.468	-	0.543	-	-	-	-	-	0.627	0.633	0.647	0.640	0.662	0.424	0.460	0.516	0.511	0.517	0.279	0.273	0.244	0.272	0.269
1	-	-	-	-	-	0.188	0.217	0.191	0.173	0.190	0.514	0.474	0.585	0.481	0.545	-	-	-	-	-	0.588	0.613	0.501	0.578	0.552
10	0.438	0.375	0.391	0.475	0.512	0.107	0.109	0.107	0.075	0.093	0.192	0.219	0.221	0.183	0.235	0.178	0.236	0.199	0.155	0.206	-	-	-	-	-

Supplement 2

Raw data shows cell numbers detected by the automated cell counting machine after conducting trypan blue exclusion assay after 24 h treatment (**S2-Tables 1-4**). Different concentrations of four tested excipients Labrasol®, Labrafil®, Transcutol®, and Labrafac® were applied on three cancer cell lines (WM164, WM1366, and D24) and two normal cells (HaCaT and primary fibroblasts).

After cell incubation for 24 h, the excipient-containing medium was removed, and cells were trypsinized using trypsin–EDTA to obtain cell suspension. 20 µl of trypan blue dye was mixed thoroughly with 20 µl cell suspension by pipetting ups and downs and loaded into the cell counting slide. Cell numbers were detected by the automated cell counting machine.

Raw tables concerning:

Figure 5: Cell viability % obtained from trypan blue exclusion assay for the excipients after 24 hr on the tested cell lines.

Table 4: IC₅₀ values obtained from trypan blue, MTT, and CV (µg/mL)

(-) These concentrations have not been tested in some cell lines. Excipient concentrations were selected based on the sensitivity of the tested cell lines.

S2-Table 1:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrasol (µg/mL)															
0.0	2.65	2.69	2.60	6.24	6.20	6.30	2.80	2.88	2.84	5.3	5.24	5.32	0.78	0.88	0.99
0.0	3.28	3.30	3.16	-	-	-	-	-	-	-	-	-	-	-	-
0.1	2.40	2.38	2.52	5.10	6.40	6.32	3.18	3.14	3.10	4.56	4.52	4.56	0.436	0.437	0.436
0.2	3.60	2.22	2.28	6.40	5.18	5.08	2.26	2.36	2.38	2.64	2.7	2.64	0.71	0.79	0.94
0.3	1.96	1.89	1.93	-	-	-	-	-	-	-	-	-	0.6	0.76	0.66
0.5	0.68	0.71	0.83	2.08	2.24	2.40	0.85	0.87	0.89	1.01	1.066	1.032	0.32	0.37	0.45

S2-Table 2:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrafil (µg/mL)															
0.0	2.65	2.69	2.60	6.24	6.20	6.30	2.80	2.88	2.84	5.30	5.24	5.32	0.78	0.88	0.99
0.0	3.28	3.30	3.16	-	-	-	-	-	-	-	-	-	-	-	-
1	3.88	3.88	3.70	5.04	5.00	5.10	-	-	-	5.10	5.06	5.00	0.51	0.55	0.63
2	1.90	1.85	1.91	1.24	1.32	1.34	1.72	1.61	1.55	2.48	2.44	2.54	0.27	0.23	0.26
4	0.90	0.82	0.93	0.95	0.90	0.97	0.50	0.52	0.43	0.93	1.30	0.94	-	-	-
20	-	-	-	-	-	-	0.38	0.38	0.38	-	-	-	-	-	-

S2-Table 3:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Transcutol (µg/mL)															
0.0	2.78	2.74	2.74	6.24	6.20	6.30	2.80	2.88	2.84	5.30	5.24	5.32	0.78	0.88	0.99
0.0	3.28	3.30	3.16	-	-	-	-	-	-	-	-	-	-	-	-
5	2.78	2.74	2.74	4.76	4.72	4.62	1.97	1.94	3.90	3.48	3.56	3.54	0.44	0.53	0.58
10	1.17	1.12	1.14	4.00	3.80	3.72	1.97	1.99	1.99	1.32	1.69	1.26	-	-	-
20	0.80	0.81	0.80	0.47	0.38	0.41	0.38	0.38	0.38	0.09	0.09	0.06	0.09	0.06	0.07

S2-Table 4:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrafac (µg/mL)															
0.0	2.65	2.69	2.6	2.8	2.88	2.84	2.80	2.88	2.84	5.30	5.24	5.32	0.78	0.88	0.99
100	2.83	2.89	2.85	8.66	8.6	8.48	2.28	2.48	2.56	3.94	3.98	3.92	-	-	-
200	-	-	-	6.18	6.22	6.3	1.49	1.48	1.41	4.24	4.36	4.08	1.20	1.23	1.06

Supplement 3

Raw data shows cellular fluorescence generated by the Dihydrorhodamine 123 oxidation conducting Reactive Oxygen Species (ROS) assay (**S3-Tables 1-5**). After 24 hours incubation with treatment solutions, medium was removed and replaced with the 100 μ l of pre-warmed dying buffer (Hank's balanced salt solution (HBSS) containing 12 μ M DHR dye). Cells were incubated for 30 minutes at 37 °C in a humidified 5 % CO₂ atmosphere in dark. The cellular fluorescence generated by the DHR oxidation was measured at 485 nm (OD 485) using a microplate reader (Multiskan™ FC Microplate Photometer).

Hydrogen peroxide (H₂O₂) treatment was used as positive control and dying buffer with no cells as blank control.

Raw tables concerning:

Figure 6: ROS intensity after cell exposure to increasing concentrations of excipients. Excipient concentrations are plotted from 0 to high on X axis (as the applied concentrations are varied depending on their toxicity, the applied doses are referred to as (0 to some multiple of IC₅₀ concentration relative to the cell line and the tested excipient and ROS generation % on Y axis (ROS production results calculated as percentage of untreated control).

Figure 7: ROS generation in each tested cell line after 24 hr exposure to the excipients at their IC₅₀ concentration

S3-Table 1:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrasol (μ g/mL)															
0.0	0.271	0.277	0.263	0.254	0.240	0.242	0.276	0.273	0.263	0.219	0.220	0.224	0.253	0.256	0.259
1	0.332	0.349	0.337	0.296	0.291	0.288	0.316	0.301	0.302	0.286	0.292	0.279	0.294	0.288	0.292
0.5	0.328	0.314	0.338	0.277	0.277	0.278	0.302	0.309	0.319	0.284	0.297	0.277	0.286	0.287	0.284
0.3	0.313	0.300	0.299	0.271	0.277	0.270	0.305	0.319	0.328	0.271	0.272	0.265	0.284	0.269	0.277
0.1	0.293	0.301	0.297	0.267	0.261	0.262	0.294	0.297	0.290	0.202	0.257	0.263	0.281	0.282	0.275

S3-Table 2:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrafil (µg/mL)															
0.0	0.276	0.274	0.251	0.243	0.236	0.222	0.273	0.251	0.224	0.211	0.219	0.220	0.257	0.248	0.222
10	0.361	0.392	0.342	0.298	0.300	0.295	0.376	0.387	0.363	-	-	-	0.276	0.268	0.232
5	0.306	0.307	0.299	0.268	0.273	0.282	0.308	0.318	0.321	0.314	0.294	0.296	0.299	0.295	0.246
2	0.293	0.306	0.298	0.253	0.259	0.259	0.302	0.303	0.305	0.289	0.299	0.276	0.309	0.300	0.243
1	-	-	-	-	-	-	-	-	-	0.280	0.281	0.283	-	-	-
0.2	0.295	0.297	0.291	0.242	0.256	0.245	0.291	0.293	0.292	-	-	-	0.281	0.282	0.283

S3-Table 3:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Transcutol (µg/mL)															
0.0	0.269	0.269	0.270	0.241	0.238	0.238	0.244	0.250	0.262	0.211	0.219	0.220	0.294	0.313	0.301
50	0.338	0.326	0.326	0.286	0.273	0.287	0.310	0.321	0.312	0.279	0.292	0.270	0.315	0.327	0.306
20	0.316	0.311	0.315	0.288	0.292	0.289	0.316	0.316	0.316	0.274	0.287	0.279	0.296	0.306	0.299
10	0.299	0.295	0.294	0.258	0.264	0.277	0.305	0.305	0.308	0.262	0.266	0.268	0.290	0.293	0.281
5	0.283	0.288	0.285	0.257	0.263	0.265	0.266	0.270	0.270	0.268	0.253	0.261	0.273	0.290	0.292

S3-Table 4:

Cell line	WM164			WM1366			D24			HaCaT			Fibroblast		
Labrafac (µg/mL)															
0.0	0.244	0.271	0.275	0.225	0.250	0.252	0.267	0.269	0.271	0.219	0.220	0.224	0.226	0.244	0.237
200	-	-	-	-	-	-	-	-	-	-	-	-	0.360	0.365	0.361
100	0.287	0.314	0.305	0.295	0.290	0.288	0.345	0.338	0.331	0.317	0.313	0.378	0.360	0.353	0.363
50	0.284	0.292	0.288	0.275	0.277	0.258	0.316	0.314	0.315	0.281	0.319	0.364	0.290	0.347	0.354
10	0.247	0.295	0.292	0.253	0.273	0.265	0.289	0.306	0.308	0.310	0.326	0.284	0.223	0.277	0.286

S3-Table 5:

Cell line		WM164			WM1366			D24			HaCaT			Fibroblast		
H2O2 (µM)	Positive control (µg/mL)															
0.0	0.0	0.254	0.259	0.267	0.231	0.230	0.223	0.243	0.250	0.227	0.219	0.220	0.224	0.226	0.244	0.237
0.0	0.0	0.258	0.264	0.251	0.227	0.230	0.225	0.230	0.229	0.239	-	-	-	0.253	0.256	0.259
0.0	0.0	0.260	0.261	0.257	0.223	0.228	0.223	0.292	0.225	0.223	-	-	-	0.257	0.248	0.222
0.0	0.0	0.260	0.258	0.254	0.228	0.220	0.219	0.227	0.231	0.275	-	-	-	-	-	-
800	27.0	-	-	-	-	-	-	-	-	-	0.281	0.291	0.271	0.290	0.288	0.280
500	17.0	0.315	0.301	0.296	-	-	-	-	-	-	-	-	-	-	-	-
400	13.5	0.315	0.301	0.296	0.281	0.273	0.275	0.306	0.304	0.300	0.253	0.261	0.269	0.286	0.2924	0.2854
200	6.75	0.297	0.297	0.297	0.269	0.273	0.275	0.293	0.283	0.300	0.259	0.231	0.241	0.242	0.246	0.243
100	3.4	0.302	0.301	0.251	0.265	0.258	0.268	0.294	0.304	0.297	-	-	-	-	-	-
50	1.7	0.248	0.292	0.280	0.261	0.233	0.227	0.277	0.270	0.280	-	-	-	-	-	-

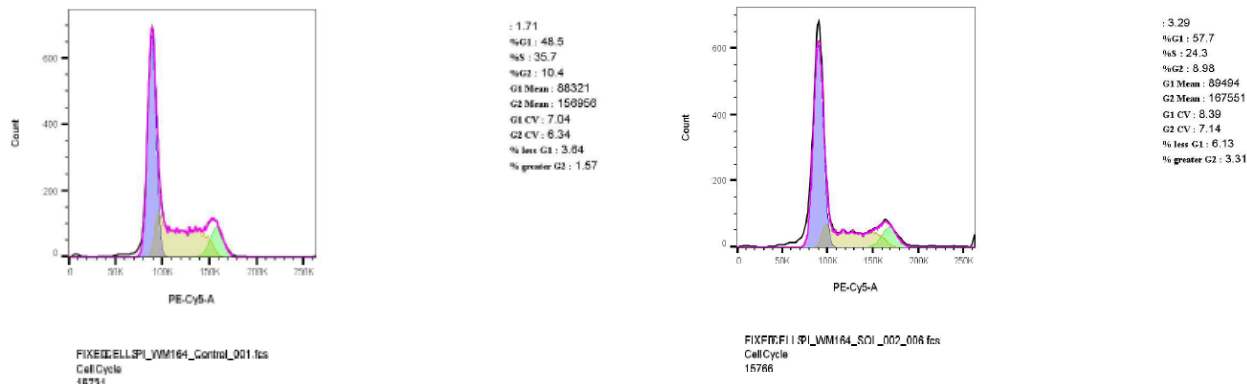
Supplement 4

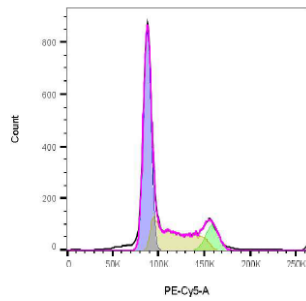
Raw data shows cell cycle histograms with DNA content in each phase of cell cycle obtained from FACS machine according to the instructions of the manufacturer. The percentages of cells in each cell-cycle phase were analyzed using FlowJo software (v10.6.1). The PI staining was used to determine the DNA content and the cell-cycle phase distributions. Attached and floating cells were collected and fixed followed by PI staining and analyzed with FACS machine according to the instructions of the manufacturer. The percentages of cells in each cell-cycle phase were analyzed using FlowJo software (v10.6.1).

Raw tables concerning:

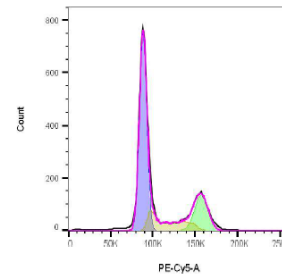
Table 5: DNA distribution (%) during cell cycle after 24-h treatment with the tested excipients

Figure 8: The effects of excipients on the process of the cell cycle of human melanoma and non-melanoma cells. The FACS diagram of the cell cycle. PE-Cy5 channel indicates the fluorescent intensity of PI, and the Y axis indicates cell number (events).





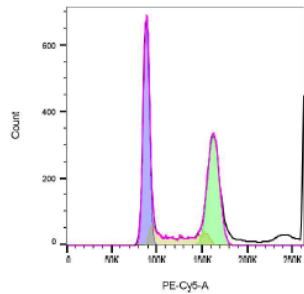
: 2.78
 %G1 : 51.7
 %S : 29.2
 %G2 : 10.4
 G1 Mean : 87339
 G2 Mean : 157940
 G1 CV : 6.63
 G2 CV : 6.47
 % less G1 : 4.97
 % greater G2 : 3.28



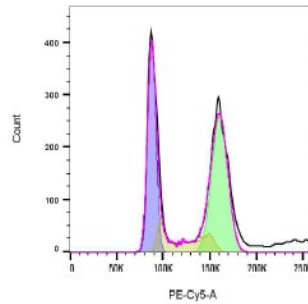
: 2.59
 %G1 : 56.4
 %S : 16.9
 %G2 : 18.7
 G1 Mean : 88143
 G2 Mean : 156720
 G1 CV : 6.99
 G2 CV : 7.26
 % less G1 : 3.41
 % greater G2 : 2.73

FIXED_F1 | 1 | WM164_F11_02_009.fcs
 CellCycle
 17532

FIXED_F1 | 1 | WM164_TR_05_002.fcs
 CellCycle
 15492



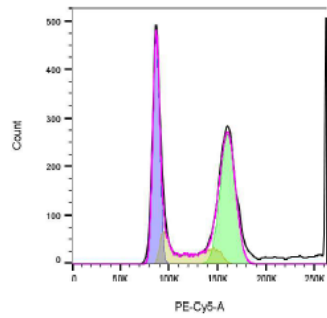
: 16.8
 %G1 : 36.4
 %S : 13.2
 %G2 : 32.6
 G1 Mean : 87943
 G2 Mean : 162206
 G1 CV : 5.33
 G2 CV : 5.35
 % less G1 : -0.42
 % greater G2 : 16.8



: 16.1
 %G1 : 30.8
 %S : 12.7
 %G2 : 38.5
 G1 Mean : 87406
 G2 Mean : 159894
 G1 CV : 7.12
 G2 CV : 7.54
 % less G1 : -0.53
 % greater G2 : 15.8

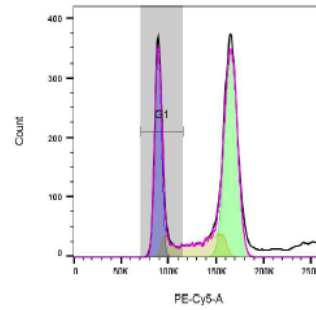
FIXED_CELL_W1366_Control_001_014.fcs
 CellCycle
 16093

FIXED_CELL_W1366_SQ1_0025_001_033.fcs
 CellCycle
 15587



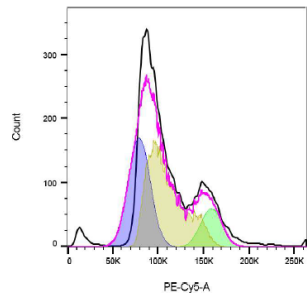
FIXE00ELLB_WM1366_FIL_02_001_021.fcs
CellCycle
15289

: 19.1
%G1 : 29.6
%S : 14.4
%G2 : 36.7
G1 Mean : 88517
G2 Mean : 160106
G1 CV : 5.74
G2 CV : 6.81
% less G1 : 0.72
% greater G2 : 15.3



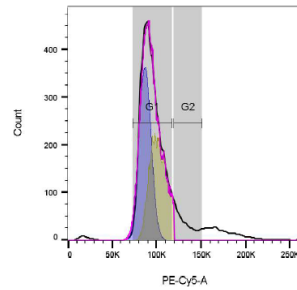
FIXE00ELLB_WM1366_TR_05_001_015.fcs
CellCycle
15769

: 47.5
%G1 : 20.8
%S : 13.3
%G2 : 38.7
G1 Mean : 88885
G2 Mean : 165010
G1 CV : 5.41
G2 CV : 5.51
% less G1 : -0.011
% greater G2 : 22.9



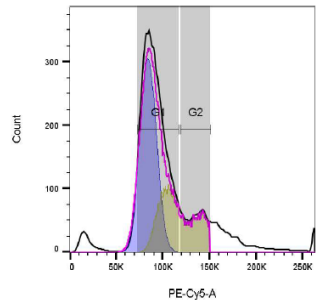
FIXE00ELLB_FB_Control_060.fcs
CellCycle
15616

: 7.37
%G1 : 39.5
%S : 52.2
%G2 : 13.5
G1 Mean : 78949
G2 Mean : 158060
G1 CV : 21.5
G2 CV : 10.4
% less G1 : -10.2
% greater G2 : 3.21



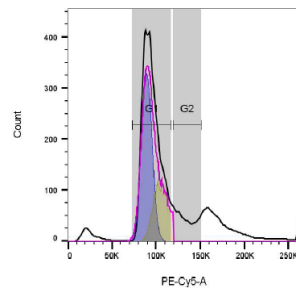
FIXE00ELLB_FB_SOL_003_062.fcs
CellCycle
14713

: 5.64
%G1 : 47.3
%S : 40.1
%G2 : 0
G1 Mean : 86536
G2 Mean : 119280
G1 CV : 11.3
G2 CV : 0
% less G1 : -0.69
% greater G2 : 17.1



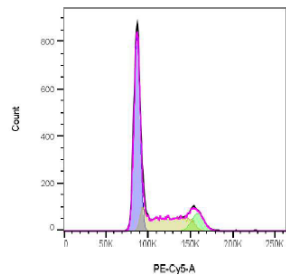
: 4.68
 %G1 : 51.9
 %S : 31.5
 %G2 : 0
 G1 Mean : 83940
 G2 Mean : 150121
 G1 CV : 15.3
 G2 CV : 0
 % less G1 : 2.43
 % greater G2 : 11.7

FIXE00ELL01_FB_FIL_02_061.fcs
 CellCycle
 15228



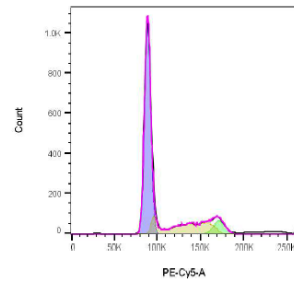
: 7.48
 %G1 : 45.1
 %S : 22.1
 %G2 : 0
 G1 Mean : 89119
 G2 Mean : 119280
 G1 CV : 9.73
 G2 CV : 0
 % less G1 : 2.34
 % greater G2 : 28.8

FIXE00ELL01_FB_TR_2_063.fcs
 CellCycle
 14130



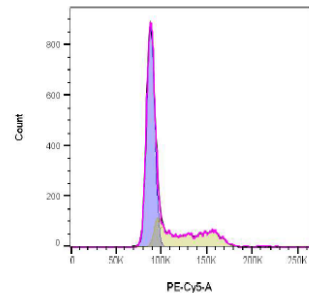
: 1.70
 %G1 : 56.3
 %S : 30.2
 %G2 : 10.3
 G1 Mean : 88530
 G2 Mean : 158246
 G1 CV : 5.72
 G2 CV : 6.49
 % less G1 : 0.50
 % greater G2 : 1.72

FIXE00F1101_HACAT_Control_048.fcs
 CellCycle
 14081



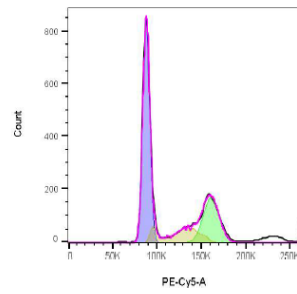
: 3.75
 %G1 : 60.7
 %S : 23.9
 %G2 : 7.14
 G1 Mean : 88546
 G2 Mean : 171722
 G1 CV : 5.60
 G2 CV : 5.39
 % less G1 : 0.63
 % greater G2 : 5.81

FIXE00F1101_HACAT_S01_002_050.fcs
 CellCycle
 16118



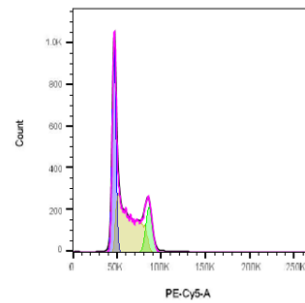
FIXF06F11_01_HACAT_F11_02_05? fcs
CellCycle
14857

: 1.54
%G1 : 60.4
%S : 38.9
%G2 : 0
G1 Mean : 88040
G2 Mean : 1.09E6
G1 CV : 6.99
G2 CV : 0
% less G1 : 0.024
% greater G2 : 0



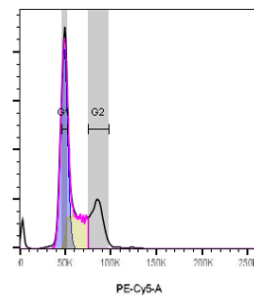
FIXF06F11_01_HACAT_TR_05_054 fcs
CellCycle
16918

: 5.11
%G1 : 50.2
%S : 16.4
%G2 : 23.1
G1 Mean : 87682
G2 Mean : 160583
G1 CV : 6.20
G2 CV : 7.87
% less G1 : 0.27
% greater G2 : 8.14



D24_Control
cell Cycle

RMSD : 1.57
%G1 : 36.4
%S : 46.2
%G2 : 13.5
G1 Mean : 46324
G2 Mean : 85708
G1 CV : 6.30
G2 CV : 5.84
% less G1 : 1.29
% greater G2 : 2.58



D24_SOL
cell Cycle

RMSD : 12.8
%G1 : 50.9
%S : 21.8
%G2 : 0
G1 Mean : 48430
G2 Mean : 75000
G1 CV : 10.7
G2 CV : 0
% less G1 : 4.88
% greater G2 : 23.0

