

### ***Supplementary Table 1: Detailed scoring of exemplary DNT tests for readiness***

Detailed scoring of the test methods summarized in Table 3. Each of the test methods were scored according to the criteria in Table 2. In the first column the short form of the criteria as in Table 2 are given. The next columns contain the scores for all the given test methods as detailed in the header of the columns. The bold interim lines give the sum of the scores for each of the 13 main categories. This score was then summarized in Table 3. UKN1 - PSC differentiation into NPC/NSC, NPC1 – hNPC proliferation, NPC2 – hNPC migration, NPC3 – hNPC neuronal differentiation, NPC4 – hNPC differentiated neurons, NPC5 – hNPC oligodendrocyte differentiation, NPC6 – hNPC oligodendrocyte maturation and TH disruption, UKN2 - NCC proliferation and migration, Embryonic phase, differentiation: MESn - Morphological ESC to neurons, Foetal phase: UKN4 (NeuriTox) –Neurite outgrowth, UKN5 (PeriTox) – Neurite outgrowth peripheral neurons, NSR neuronal subtype ratio Neuronal maturation: Syn – Synaptogenesis, Nnff – Neuronal Network formation and function, 3Dr - Astrocytes, Oligodendrocytes, Myelination, Microglia in 3D rat, 3Dh - Astrocytes, Oligodendrocytes, Myelination, Microglia in 3D human,ZFE - Zebrafish

Criteria	Max. score	UKN1	NPC1	NPC2	NPC3	NPC4	NPC5	NPC6	UKN2	MESn	UKN4 NeuriTox	UKN5 PeriTox	NSR	SYN	Nnff	3Dr	3Dh	ZFE	
<b>1 Test system</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>10</b>	<b>9</b>	<b>7</b>	<b>10</b>	<b>9</b>	<b>4</b>	<b>8</b>	<b>8</b>	<b>10</b>	<b>7</b>	<b>8</b>	
1a What is modelled	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
1b Relevance	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
1c System uncertainties and human correlate (HC)	1	1	1	1	1	1	1	1	0	0	1	0	0	0	0.5	1	1	1	
1d Definition of cells	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	
1e Cell composition	1	1	1	1	1	1	1	1	1	0.5	1	1	1	1	1	1	1	1	
1f Cellular environment	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	
1g Biological consistency	1	1	1	1	1	1	1	1	1	0.5	1	1	0	1	0.5	1	0	0	
1h Critical components	1	1	1	1	1	1	1	1	1	0.5	1	1	0	1	1	1	0	0	
1i Cell stability	1	1	1	1	1	1	1	1	1	0.5	1	1	0	1	1	1	0	1	
1j <i>Transgenic cells</i>	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	
<b>2 Exposure scheme</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2.5</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	
2a Description	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	
2b Unique identity	1	1	1	1	1	1	1	1	1	0.5	1	1	0	1	1	1	1	1	
2c Graphical scheme	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
<b>3 Documentation / SOP</b>	<b>5</b>	<b>3</b>	<b>5</b>	<b>4.5</b>	<b>4.5</b>	<b>4.5</b>	<b>4.5</b>	<b>4.5</b>	<b>5</b>	<b>0.5</b>	<b>5</b>	<b>5</b>	<b>3.5</b>	<b>3</b>	<b>3</b>	<b>5</b>	<b>2</b>	<b>4</b>	
3a <i>Availability</i>	1	1	1	1	1	1	1	1	1	0.5	1	1	1	1	1	1	0	1	
3b Stage of development	1	0	1	1	1	1	1	1	1	0	1	1	1	0	0	1	0	0	
3c <i>For CRO tests</i>	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0.5	1	0	1	
3d Test components	1	0	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1	
3e Stocks	1	1	1	0.5	0.5	0.5	0.5	0.5	0.5	1	0	1	1	0.5	1	0.5	1	1	
<b>4 Main endpoint(s)</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>2.5</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>3</b>
4a Biol. relevance	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
4b Toxicol. relevance	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
4c Analytical methods	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	0	
4d Multiple endpoints	1	1	1	1	1	1	1	1	1	0.5	1	1	0	1	1	1	1	1	
<b>5 Cytotoxicity</b>	<b>5</b>	<b>4</b>	<b>4</b>	<b>4.5</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>5</b>	<b>1</b>	<b>5</b>	<b>5</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>2.5</b>	<b>3.5</b>	
5a Cytotoxicity within test	1	1	1	1	1	1	1	1	1	0	1	1	1	0.5	1	1	1	1	
5b Subpopulation effects	0.5	0	0	0.5	0	0	0	0	0.5	0	0.5	0.5	1	0	0	0	0	0.5	
5c Specificity (compared to cytotox)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5	0	0.5	0.5	0.5	0.5	0	
5d <i>Timing within test</i>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5	0	0	0.25	0.5	0	0.5	
5e <i>Timing after test</i>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0.25	0.5	0	0.5	
5f Curve fitting	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.25	0.5	0	0.5	0.5	0.5	0.5	0.5	0.5	0	0	
5g Non-cytotoxicity	0.5	0	0.25	0.25	0	0	0	0	0.5	0	0.5	0.5	0.5	0.5	0.25	0	0	0	
5h Bench mark response	0.5	0.5	0.25	0.25	0.5	0.5	0.5	0.5	0.5	0	0.5	0.5	0	0	0.25	0.5	0.5	0.5	
5i <i>Apoptosis/ Proliferation</i>	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0	0	0	0.5	0.5	0.5	
<b>6 Test method controls</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>1.5</b>	<b>4</b>	<b>3.5</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>4</b>	<b>0</b>	<b>2</b>	
6a Positive controls (PC)	1	1	1	1	1	0.5	1	1	1	0	1	1	0	1	1	1	0	1	

6b Negative controls (NC)	1	1	1	1	1	0	1	0.5	1	0	1	1	0	0	0.5	1	0	0
6c Unspecific controls (UC)	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0.5	1	0	0
6d Endpoint-specific controls (EC)	1	1	1	1	1	0	1	1	1	0	1	1	0	0	1	1	0	1
<b>7 Data evaluation</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>4</b>	<b>0.5</b>	<b>4</b>	<b>3</b>	<b>1</b>	<b>1</b>	<b>2.5</b>	<b>4</b>	<b>2</b>	<b>3</b>
7a Outliers	1	1	1	1	1	1	1	1	1	0.5	1	0	0	0	0.5	1	0	1
7b Conc.-dependence	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0
7c Benchmark response	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0.5	1	1	1
7d Curve fitting	1	1	1	1	1	1	1	0	1	0.5	1	1	0	0	0.5	1	0	1
<b>8 Testing strategy</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>0</b>	<b>4</b>	<b>3</b>	<b>1</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>3</b>
8a Hazard prediction	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1
8b Link to an AOP	1	1	1	1	1	1	1	1	1	0	1	0	0	1	1	1	1	0
8c Role in battery	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1
8d Comparison to similar tests	1	1	1	1	1	1	1	1	1	0	1	1	0	1	1	1	1	1
<b>9 Robustness</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>3</b>	<b>2.5</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>1</b>	<b>2</b>	<b>3.5</b>	<b>4</b>	<b>0</b>	<b>2</b>
9a Reproducibility	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1
9b Intra-lab	1	1	1	1	1	1	1	1	1	0	1	1	0	0	1	1	0	1
9c Inter-lab	1	0	0	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0
9d Historical controls	1	1	1	1	1	1	1	0.5	1	0	1	1	0	1	0.5	1	0	0
<b>10 Test benchmarks</b>	<b>4</b>	<b>3</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>0.5</b>	<b>2.5</b>	<b>1.5</b>	<b>4</b>	<b>0</b>	<b>4</b>	<b>4</b>	<b>0</b>	<b>1</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>
10a Sensitivity (of the test)	1	0	1	1	1	0.5	0	0	1	0	1	1	0	0	1	1	0	0
10b Specificity (of the test)	1	1	1	1	1	0	0.5	0	1	0	1	1	0	0	1	1	0	1
10c Acceptance criteria	1	1	1	1	1	0	1	1	1	0	1	1	0	0	0.5	1	0	1
10d Response characteristics	1	1	1	1	1	0	1	0.5	1	0	1	1	0	1	0.5	1	0	1
<b>11 Prediction model</b>	<b>4</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>4</b>	<b>2</b>	<b>0</b>	<b>0</b>	<b>2.5</b>	<b>4</b>	<b>0</b>	<b>1</b>
11a Definition	1	1	1	1	1	0	1	1	1	0	1	1	0	0	1	1	0	1
11b Rationale	1	1	0.5	1	1	0	1	1	1	0	1	1	0	0	0.5	1	0	0
11c Confirmation	1	0	0.5	1	1	0	1	1	0	0	1	0	0	0	0.5	1	0	0
11d Limitations	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0.5	1	0	0
<b>12 Applicability domains</b>	<b>3</b>	<b>2</b>	<b>2</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2.5</b>	<b>2.5</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>1</b>	<b>0</b>	<b>3</b>	<b>2</b>	<b>3</b>	<b>2</b>	<b>1</b>
12a Chemicals	1	1	0.5	1	1	1	0.5	0.5	1	0	1	1	0	1	0.5	1	1	1
12b Pathways	1	1	0.5	1	1	1	1	1	0	0	1	0	0	1	0.5	1	0	0
12c AOP	1	0	1	1	1	1	1	1	0	0	1	0	0	1	1	1	1	0
<b>13 Screening hits</b>	<b>4</b>	<b>3</b>	<b>3</b>	<b>3</b>	<b>2</b>	<b>1</b>	<b>2</b>	<b>2</b>	<b>4</b>	<b>0</b>	<b>3</b>	<b>3</b>	<b>0</b>	<b>2</b>	<b>2.5</b>	<b>4</b>	<b>1</b>	<b>4</b>
13a Hit definition	1	1	0	0	0	0	0	0	1	0	1	1	0	1	0.5	1	1	1
13b Hit confirmation (prim.)	1	1	1	1	1	0.5	1	1	1	0	1	1	0	1	0.5	1	0	1
13c Hit confirmation (sec.)	1	0	1	1	0	0	0	0	1	0	0	0	0	0	0.5	1	0	1
13d Screen documentation	1	1	1	1	1	0.5	1	1	1	0	1	1	0	0	1	1	0	1