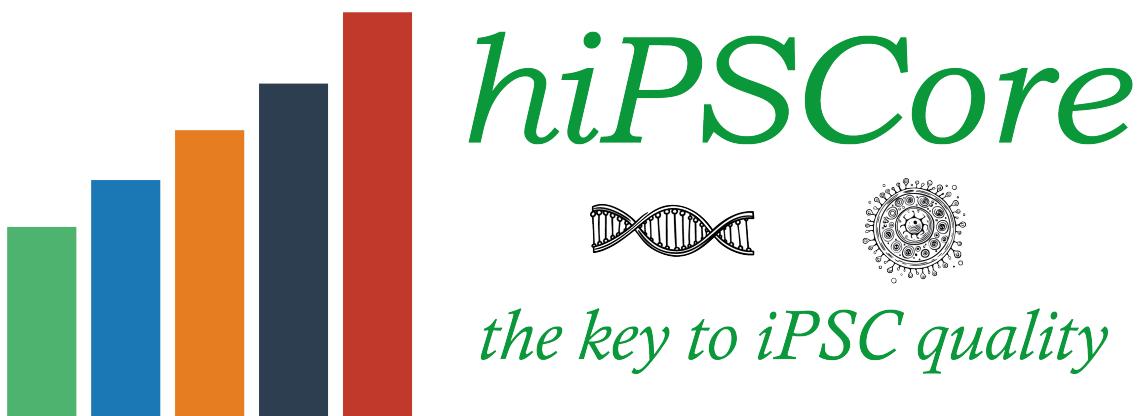


# hiPSCore Report DU411 - 2025-06-03



The hiPSCore is a machine learning-based thoroughly validated functional pluripotency test. By analyzing hiPSC-derived undifferentiated, endoderm-, ectoderm-, and mesoderm-differentiated samples using qPCR, the automated hiPSCore classification assesses differentiation potential of hiPSCs with high accuracy, sensitivity, and specificity. Successfully validated cell lines are truly pluripotent according to the Standards of the International Society for Stem Cell Research (ISSCR) and may be used for research. The following report contains detailed analysis and description of the results.

## **Report Parameters**

### **Selected Parameters**

These parameters were selected to be included in the report:

- hiPSCore plot
- hiPSCore GL plot
- Sample Summary
- Mean Ct table
- Mean dCt table
- hiPSCore subtest table
- hiPSCore GL table
- hiPSCore Duo Score

These samples were analyzed:

- DU411\_DMD\_D2\_meso
- DU411\_DMD\_D2\_Ecto
- DU411\_DMD\_D2\_Endo
- DU411\_DMD\_D2\_undiff

Scoring Model Version: **0.3**

# Report

## hiPSCore Results Plot

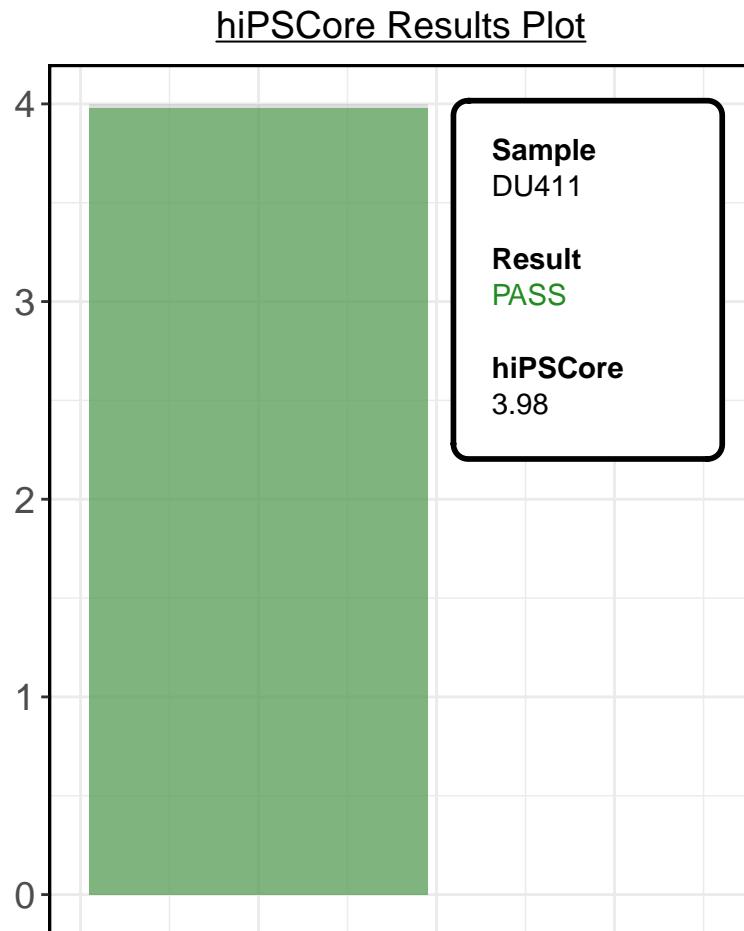


Figure 1: This graph shows the results of the canonical hiPSCore classification model based on the classification of iPSC-derived undifferentiated, endoderm, ectoderm, and mesoderm samples. A score above 3 indicates successful individual subtests and validate the tested iPSC lines functional pluripotency, i.e. the capacity of undifferentiated iPSCs to differentiate into each of the three primary germ layers.

## hiPSCore Cell Fate Results Plot

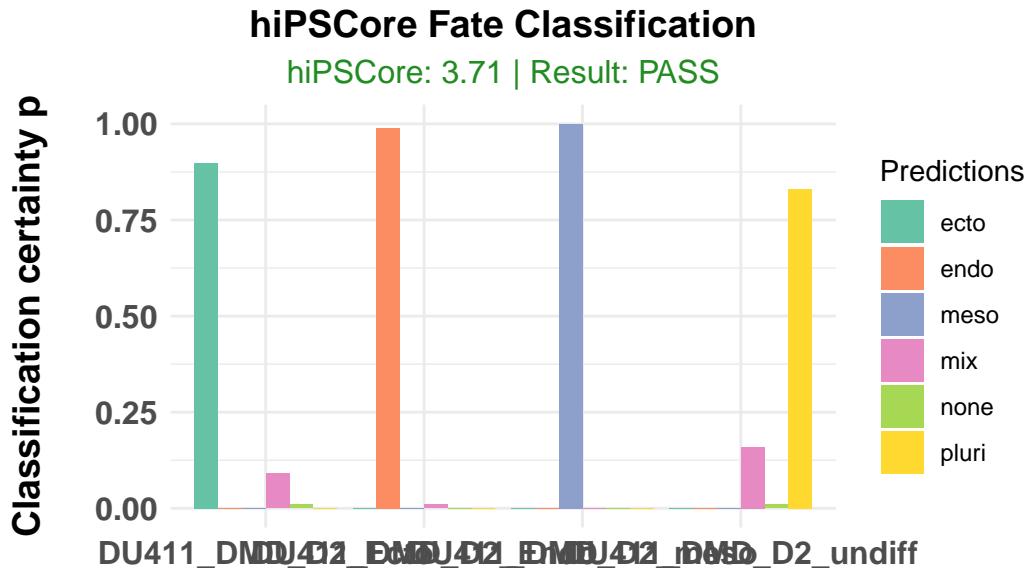


Figure 2: This graph shows the results of the hiPSCore GL/Cell Fate classification model. Here, samples are classified as either undifferentiated iPSCs, endoderm, ectoderm, mesoderm, or none of the before, or a mix of the before. The certainty p of classification is given for each sample with high values above 0.8 indicating high quality pure samples. Lower values or high values for none or mix classes indicate bad sample quality and warrant reanalysis of the samples.

## Sample Summary

The sample summary indicates individual per sample quality and takes into the account both the cell fate classification and the cell fate-specific subtest. Only if both are consistent a sample is regarded as good quality!

DU411_DMD_D2_Ect	DU411_DMD_D2_End	DU411_DMD_D2_meso	DU411_DMD_D2_undiff
is ecto with a certainty of 0.89. This represents a high confidence/likelihood that the classification is correct. The corresponding subtest (ecto) has a certainty p of 1. This represents a high confidence/likelihood that	is endo with a certainty of 0.99. This represents a high confidence/likelihood that the classification is correct. The corresponding subtest (endo) has a certainty p of 1. This represents a high confidence/likelihood that	is meso with a certainty of 1. This represents a high confidence/likelihood that the classification is correct. The corresponding subtest (meso) has a certainty p of 1. This represents a high confidence/likelihood that	is pluri with a certainty of 0.83. This represents a medium confidence/likelihood that the classification is correct. The corresponding subtest (pluri) has a certainty p of 0.98. This represents a high confidence/likelihood that
DU411_DMD_D2_Ect contains cells of the origin ecto. The combined hiPSCore score is 1.89 indicating Good Quality of your Sample. For details consult the individual tables generated.	DU411_DMD_D2_End contains cells of the origin endo. The combined hiPSCore score is 1.99 indicating Good Quality of your Sample. For details consult the individual tables generated.	DU411_DMD_D2_meso contains cells of the origin meso. The combined hiPSCore score is 2 indicating Good Quality of your Sample. For details consult the individual tables generated.	DU411_DMD_D2_undiff contains cells of the origin pluri. The combined hiPSCore score is 1.81 indicating Good Quality of your Sample. For details consult the individual tables generated.

## Report Tables – Underlying Data & Analysis

### hiPSCore Germlayer Table

This table displays the results of the hiPSCore germlayer model which classifies cell types according to their cell fate as either undifferentiated iPSCs, endoderm, ectoderm, mesoderm, or none of the before, or a mix of the before. The prediction\_val indicates the top, i.e. most likely cell fate of analyzed samples. Individual predicton values for each of the six classes (pluri = undifferentiated iPSCs, endo = endoderm, ecto = ectoderm, meso = mesoderm, none = neither of the first four, mix = mix of two or more of the first four) are given and indicate the likelihood of a prediction to be correct or not. High ( $\geq 0.8$ ) values indicate high certainty of classification whereas medium ( $\geq 0.5 \& \leq 0.8$ ) and low ( $\geq 0.49$ ) values indicate that an analyzed sample is not pure and of overall bad quality.

Sample	.pred	pluri	endo	ecto	meso	none	mix
DU411_DMD_D2_Ecto	ecto	0.00	0.00	0.89	0	0.01	0.09
DU411_DMD_D2_Endo	endo	0.00	0.99	0.00	0	0.00	0.01
DU411_DMD_D2_meso	meso	0.00	0.00	0.00	1	0.00	0.00
DU411_DMD_D2_undiff	pluri	0.83	0.00	0.00	0	0.01	0.16

### hiPSCore duo Score Table

This table shows the result of the hiPSCore duo score which is an enhanced stricter implementation combining both the power of the multi-class classification and the binary classification backing the individual subtests. The forward implementation first classifies the cell fate and then backs this classification up by performing the respective subtest. The result is a continuous score between 0 and 2, with  $\geq 1.6$  indicating a good quality sample, between 1.3 and 1.6 issuing a low score flag, between 1.1 and 1.3 issuing a warning and  $\leq 1.1$  indicating a sample of bad quality.

sample_ID	pred.	gl_p	subtest_p	duo_hiPSCore	FLAG
DU411_DMD_D2_meso	meso	1.00	1.00	2.00	PASS
DU411_DMD_D2_Ecto	ecto	0.89	1.00	1.89	PASS
DU411_DMD_D2_Endo	endo	0.99	1.00	1.99	PASS
DU411_DMD_D2_undiff	pluri	0.83	0.98	1.81	PASS

### Mean $C_t$ Table

This table displays the mean  $C_t$  values ( $\pm \sigma$ ) of each gene target for each sample and gives an indication of the qPCR run quality.

Sample Name	Target Name	mean	sd
DU411_DMD_D2_Ecto	ACTB	16.7	0.1
DU411_DMD_D2_Ecto	APLNR	28.1	0.4
DU411_DMD_D2_Ecto	CER1	31.9	0.1
DU411_DMD_D2_Ecto	CNMD	28.6	0.1
DU411_DMD_D2_Ecto	EOMES	33.0	1.4
DU411_DMD_D2_Ecto	GAPDH	16.8	0.0
DU411_DMD_D2_Ecto	GATA6	30.5	0.6
DU411_DMD_D2_Ecto	HAND1	36.9	4.3
DU411_DMD_D2_Ecto	HES5	28.4	0.3
DU411_DMD_D2_Ecto	HOXB7	31.8	0.2
DU411_DMD_D2_Ecto	NANOG	29.0	0.4

Sample Name	Target Name	mean	sd
DU411_DMD_D2_Ecto	PAMR1	23.1	0.1
DU411_DMD_D2_Ecto	PAX6	23.3	0.0
DU411_DMD_D2_Ecto	SPP1	27.7	0.1
DU411_DMD_D2_Endo	ACTB	16.6	0.0
DU411_DMD_D2_Endo	APLNR	27.3	0.0
DU411_DMD_D2_Endo	CER1	16.7	0.0
DU411_DMD_D2_Endo	CNMD	30.3	0.5
DU411_DMD_D2_Endo	EOMES	20.8	0.0
DU411_DMD_D2_Endo	GAPDH	18.0	0.0
DU411_DMD_D2_Endo	GATA6	20.5	0.0
DU411_DMD_D2_Endo	HAND1	27.1	0.5
DU411_DMD_D2_Endo	HES5	32.9	1.8
DU411_DMD_D2_Endo	HOXB7	33.4	0.2
DU411_DMD_D2_Endo	NANOG	24.3	0.1
DU411_DMD_D2_Endo	PAMR1	26.8	0.0
DU411_DMD_D2_Endo	PAX6	26.9	0.1
DU411_DMD_D2_Endo	SPP1	28.6	0.4
DU411_DMD_D2_meso	ACTB	17.7	0.1
DU411_DMD_D2_meso	APLNR	22.0	0.0
DU411_DMD_D2_meso	CER1	31.2	0.8
DU411_DMD_D2_meso	CNMD	28.4	0.0
DU411_DMD_D2_meso	EOMES	33.3	1.0
DU411_DMD_D2_meso	GAPDH	17.9	0.0
DU411_DMD_D2_meso	GATA6	28.6	0.1
DU411_DMD_D2_meso	HAND1	25.3	0.1
DU411_DMD_D2_meso	HES5	31.7	0.2
DU411_DMD_D2_meso	HOXB7	25.6	0.0
DU411_DMD_D2_meso	NANOG	31.7	0.6
DU411_DMD_D2_meso	PAMR1	31.5	0.4
DU411_DMD_D2_meso	PAX6	40.0	0.0
DU411_DMD_D2_meso	SPP1	29.1	1.1
DU411_DMD_D2_undiff	ACTB	15.4	0.0
DU411_DMD_D2_undiff	APLNR	28.6	0.3
DU411_DMD_D2_undiff	CER1	25.8	0.1
DU411_DMD_D2_undiff	CNMD	23.2	0.0
DU411_DMD_D2_undiff	EOMES	26.2	0.0
DU411_DMD_D2_undiff	GAPDH	16.3	0.0
DU411_DMD_D2_undiff	GATA6	30.4	0.3
DU411_DMD_D2_undiff	HAND1	28.2	0.2
DU411_DMD_D2_undiff	HES5	30.5	0.4
DU411_DMD_D2_undiff	HOXB7	31.1	0.1

Sample Name	Target Name	mean	sd
DU411_DMD_D2_undiff	NANOG	22.3	0.0
DU411_DMD_D2_undiff	PAMR1	26.1	0.2
DU411_DMD_D2_undiff	PAX6	30.2	0.2
DU411_DMD_D2_undiff	SPP1	23.1	0.1

### Mean $\Delta C_t$ Table

This table displays the mean  $\Delta C_t$  values normalized to the reference genes for each sample.

Sample Name	PAX6	SPP1	genes	dct
DU411_DMD_D2_Ecto	6.551150	11.031949	APLNR	11.36
DU411_DMD_D2_Ecto	6.551150	11.031949	CER1	15.18
DU411_DMD_D2_Ecto	6.551150	11.031949	CNMD	11.89
DU411_DMD_D2_Ecto	6.551150	11.031949	EOMES	16.28
DU411_DMD_D2_Ecto	6.551150	11.031949	GATA6	13.77
DU411_DMD_D2_Ecto	6.551150	11.031949	HAND1	20.21
DU411_DMD_D2_Ecto	6.551150	11.031949	HES5	11.66
DU411_DMD_D2_Ecto	6.551150	11.031949	HOXB7	15.10
DU411_DMD_D2_Ecto	6.551150	11.031949	NANOG	12.28
DU411_DMD_D2_Ecto	6.551150	11.031949	PAMR1	6.38
DU411_DMD_D2_Endo	9.557967	11.293513	APLNR	10.03
DU411_DMD_D2_Endo	9.557967	11.293513	CER1	-0.60
DU411_DMD_D2_Endo	9.557967	11.293513	CNMD	13.02
DU411_DMD_D2_Endo	9.557967	11.293513	EOMES	3.52
DU411_DMD_D2_Endo	9.557967	11.293513	GATA6	3.14
DU411_DMD_D2_Endo	9.557967	11.293513	HAND1	9.81
DU411_DMD_D2_Endo	9.557967	11.293513	HES5	15.63
DU411_DMD_D2_Endo	9.557967	11.293513	HOXB7	16.09
DU411_DMD_D2_Endo	9.557967	11.293513	NANOG	6.95
DU411_DMD_D2_Endo	9.557967	11.293513	PAMR1	9.48
DU411_DMD_D2_meso	22.182827	11.318229	APLNR	4.19
DU411_DMD_D2_meso	22.182827	11.318229	CER1	13.34
DU411_DMD_D2_meso	22.182827	11.318229	CNMD	10.55
DU411_DMD_D2_meso	22.182827	11.318229	EOMES	15.49
DU411_DMD_D2_meso	22.182827	11.318229	GATA6	10.73
DU411_DMD_D2_meso	22.182827	11.318229	HAND1	7.49
DU411_DMD_D2_meso	22.182827	11.318229	HES5	13.91
DU411_DMD_D2_meso	22.182827	11.318229	HOXB7	7.83
DU411_DMD_D2_meso	22.182827	11.318229	NANOG	13.87

Sample Name	PAX6	SPP1	genes	dct
DU411_DMD_D2_meso	22.182827	11.318229	PAMR1	13.71
DU411_DMD_D2_undiff	14.406999	7.244837	APLNR	12.75
DU411_DMD_D2_undiff	14.406999	7.244837	CER1	10.00
DU411_DMD_D2_undiff	14.406999	7.244837	CNMD	7.36
DU411_DMD_D2_undiff	14.406999	7.244837	EOMES	10.40
DU411_DMD_D2_undiff	14.406999	7.244837	GATA6	14.53
DU411_DMD_D2_undiff	14.406999	7.244837	HAND1	12.40
DU411_DMD_D2_undiff	14.406999	7.244837	HES5	14.66
DU411_DMD_D2_undiff	14.406999	7.244837	HOXB7	15.27
DU411_DMD_D2_undiff	14.406999	7.244837	NANOG	6.43
DU411_DMD_D2_undiff	14.406999	7.244837	PAMR1	10.28

### hiPSCore subtest Table

This table displays the individual hiPSCore subtest results and gives an indication on which cell types are contained within analyzed samples. A high ( $\geq 0.8$ ) X1 prediction value indicates that a given cell type is highly likely present in the analyzed sample, whereas medium ( $\geq 0.5 \& \leq 0.8$ ) and low ( $\leq 0.49$ ) values indicate that a given cell type is unlikely to be present in the analyzed sample.

X0	X1	Sample	test	eval
1.00	0.00	DU411_DMD_D2_Ecto	undifferentiated	FAIL
1.00	0.00	DU411_DMD_D2_Endo	undifferentiated	FAIL
1.00	0.00	DU411_DMD_D2_meso	undifferentiated	FAIL
0.02	0.98	DU411_DMD_D2_undiff	undifferentiated	PASS
1.00	0.00	DU411_DMD_D2_Ecto	endoderm	FAIL
0.00	1.00	DU411_DMD_D2_Endo	endoderm	PASS
1.00	0.00	DU411_DMD_D2_meso	endoderm	FAIL
1.00	0.00	DU411_DMD_D2_undiff	endoderm	FAIL
0.00	1.00	DU411_DMD_D2_Ecto	ectoderm	PASS
1.00	0.00	DU411_DMD_D2_Endo	ectoderm	FAIL
1.00	0.00	DU411_DMD_D2_meso	ectoderm	FAIL
1.00	0.00	DU411_DMD_D2_undiff	ectoderm	FAIL
1.00	0.00	DU411_DMD_D2_Ecto	mesoderm	FAIL
0.94	0.06	DU411_DMD_D2_Endo	mesoderm	FAIL
0.00	1.00	DU411_DMD_D2_meso	mesoderm	PASS
1.00	0.00	DU411_DMD_D2_undiff	mesoderm	FAIL