DMD-212109\_HEK293-HostCellProtein\_ELISA – Method History File

legacy RPT-000327

# Introduction Section

## Objective

This method history file (MHF) document represents a summary of analytical work packages to proof that the method is seen as “fit for purpose”.

The signed MHF is the report of a “fit for purpose” qualification.

## Principle of the Method

This method is used to determine HEK 293 host cell proteins (HCP) in samples using an ELISA sandwich system with a specific antibody. The HEK 293 Host Cell Protein assay is a two-site immunoenzymetric assay. Samples containing HEK 293 cell proteins are reacted in microtiter strips coated with an affinity purified capture antibody. An HRP labeled anti-HEK 293 antibody is reacted simultaneously, forming a sandwich complex of solid phase antibody-HEK 293 HCP-enzyme labeled antibody. The microtiter strips are then washed to remove any unbound reactantsPCP. After the washes, the substrate tetramethylbenzidine is then reacted. The amount of hydrolyzed substrate is read on a microtiter plate reader and is directly proportional to the concentration of HEK 293 Host cell proteins present.

For a detailed method description refer to SOP-051247.

Project: SHP654, SHP648, AAV3b, TAK-686, DP0079 (TAK-709; FVIII 2nd Gen), DP0073; FXN (Friedreich Ataxia), AAV6, Fabry

Group: Gene Therapy – Process Analytics

## Intended Use

The method is used for the following purpose:

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Fit for purpose | | | | | |  |  |  |  |
|  | Clinical Phase 1 |  | Clinical Phase 2 |  | Clinical Phase 3 |  | Conformance runs, PPQ |  | Commercial support |

## Classification of testing method

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Identification | | | | | | |
|  | Test for Impurities |  | Quantitative |  | Limit | | |
|  | Assay |  | Quantitative |  | Presence/Absence |  | Identification |
|  | not classifiable, | due to | | | | | |

# Acronyms and Definitions

Broadly used terms should be referred to the respective Shire Global Quality Standard Glossary (TO STRD-048; VV-00940858).

|  |  |
| --- | --- |
| Acronym/Definition | Description |
| Accuracy | The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. [ICH Q2(R1)] |
| Calibration | Calibration is the demonstration that a particular instrument or device produces results within specified limits by comparison with those produced by a reference or traceable standard over an appropriate range of measurements. [ICH Q7] |
| Matrix | Refers to the components of a sample other than the analyte of interest. Includes entities such as buffers, extraction media and cell culture fluids. |
| Precision | The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision may be considered at three levels: repeatability, intermediate precision and reproducibility. [ICH Q2(R1)] |
| Qualified | An individual is classified as qualified if he or she has demonstrated the ability to perform a task according to a set of defined criteria. |
| Quantitative test | A quantitative test is a test in which the amount of analyte in a sample is measured and the numerical value is reported. [Derived from ICH Q2(R1)] |
| Range | The range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated that the analytical procedure has a suitable level of precision, accuracy and linearity. [ICH Q2(R1)] |
| Reference standard | A reference standard is a substance that has been shown by an extensive set of analytical tests to be authentic material that should be of high purity. This standard can be: (1) obtained from an officially recognized source, or (2) prepared by independent synthesis, or (3) obtained from existing production material of high purity, or (4) prepared by further purification of existing production material. [ICH Q7] |
| Repeatability | Repeatability expresses the precision under the same operating conditions over a short interval of time. Repeatability is also termed intra-assay precision [ICH Q2(R1)] |
| Robustness | The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage. [ICH Q2(R1)] Robustness is typically performed during development of the method, but may be performed during validation. It is to identify the factors that should be assessed when measuring precision of a method during validation |
| System suitability | System suitability confirmatory test procedures and parameters to ensure that the system (equipment, electronics, and analytical operations and controls to be analyzed) will function correctly as an integrated system at the time of use. |

# Current Method Performance

The current method can be used for detecting HEK 293 Host Cell Proteins in samples.

Table 1: Overview of Method parameters

|  |  |
| --- | --- |
| Accuracy | Recovery of spiking samples 80%-120%  A list of all FFP samples and their composition can be found in Section 6.0 |
| Precision – Intra Assay | measured with AFF\_ELU: CV=4.9% |
| Precision – Inter Assay | measured with n=7 BDS limittests: CV=10.4% |
| Specificity | Variation <20% when using tested pre-dilutions for samples, cross signal observed for HSA (human serum albumin) |
| Detection limit | 3,125 ng/mL |
| Quantitation limit | 4ng/ml |
| Linearity | Shown for 4-100ng/ml, |
| Range | 3,125-200ng/ml; as reference curve |
| Robustness | 30 min at 37°C: 92% recovery  2h Benchtop Recovery: 103%  3 F/T-cycles Recovery: 105% |
| System Suitability Test | Photometer: yearly maintenance / quarterly check  Pipettes: Yearly / biyearly calibration |
| Equipment Qualification | Instrument maintained and calibrated, control sample as system functionality test and performance check. |

# Analytical Quality by Design (AQbD) Approach

## Target Measurement and Analytical Target Profile

The HEK293 Host Cell Protein content is determined with a coefficient of variance as low as possible but at least below 20%, as this limit is given in the FDAs Bioanalytical Method Validation, Guidance for Industry, 2018.

## Method Selection

The method was transferred from BioAnalytics / Analytical Development Vienna (see method transfer protocol DFM04834) as ELISAs are highly specific which is necessary to make accurate quantification of HEK293 Host Cell Proteins.

## Risk Assessment

N/A.

### Comparison testing TAK-748, Tak-754 and TAK-686 with cross-spiking (DFM09684)

A comparison testing of the projects TAK-748, Tak-754 and TAK-686 was made. Therefore, a BDS sample of each project was tested as well as a sample from a step earlier to BDS (“VS”  “Vorstufe”). Each BDS sample was then spiked 1:10 with each of the respective other sample step. The recovery of the measured value relative to the desired value was then calculated. All recoveries were within 91%-105%.

Additionally, each BDS sample was spiked 1:10 with the 200ng/ml reference aliquot. The estimated recoveries are within 91%-99%.

|  |  |
| --- | --- |
| **Sample Name** | **Recovery [%]** |
| PP754\_1905TMAE\_FILT (corresponds BDS) + PP954 VS Spike | 95 |
| PP754\_1905TMAE\_FILT (corresponds BDS) + PP648 VS Spike | 97 |
| PP754\_1905TMAE\_FILT (corresponds BDS) + PP686 VS Spike | 99 |
| PP648\_1819TMAE\_FILT (corresponds BDS) + PP954 VS Spike | 91 |
| PP648\_1819TMAE\_FILT (corresponds BDS) + PP648 VS Spike | 105 |
| PP648\_1819TMAE\_FILT (corresponds BDS) + PP686 VS Spike | 97 |
| PP686\_1912POL\_FLT (corresponds BDS) + PP954 VS Spike | 91 |
| PP686\_1912POL\_FLT (corresponds BDS) + PP648 VS Spike | 103 |
| PP686\_1912POL\_FLT (corresponds BDS) + PP686 VS Spike | 99 |
| PP754\_1905TMAE\_FILT (corresponds BDS) + Spike reference 200ng/ml | 94 |
| PP648\_1819TMAE\_FILT (corresponds BDS) + Spike reference 200ng/ml | 99 |
| PP686\_1912POL\_FLT (corresponds BDS) + Spike reference 200ng/ml | 99 |

## Method Optimization

### Internal reference preparation HV6AR00 prediluted to 2000ng/ml and aliquoted (#190912/HV6AR00 – 2000ng/ml) (DFM10425)

For preparation of an internal reference, original concentrated HV6AR00 vials were pooled and diluted to 2000ng/ml in Cygnus sample dilution buffer (I028). About 800 aliquots were prepared and stored at <-60°C.

For performing a test, an aliquot has to be thawed and diluted 1:10 in sample diluent buffer for a final concentration of 200ng/ml.

### Comparison Testing HV6AR00 reference versus Kit standard reference (DFM10668)

All sample results from plate 1 and plate 2, that were calculated based on HV6AR00 standard, show recoveries of 80%-120% relatively to the kit standard results.

The back calculated values of the HV6AR00 reference are within 80%-120% recovery except plate 2 and plate 4 for the Std8 (1,5625ng/ml).

Plate 4 as example of back calculated HV6AR00 reference values:

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Desired values [ng/ml]** | **OD** | **Back calculated from OD values [%]** |
| Std1 | 200 | 1,973 | 100 |
| Std2 | 100 | 1,218 | 100 |
| Std3 | 50 | 0,673 | 98 |
| Std4 | 25 | 0,365 | 100 |
| Std5 | 12,5 | 0,19 | 101 |
| Std6 | 6,25 | 0,099 | 104 |
| Std7 | 3,125 | 0,049 | 101 |
| Std8 | 1,5625 | 0,019 | 76 |

### Implementation Testing of HV6AR00 reference (DFM10919)

For implementation in routine testing, the standard range is narrowed down to 200ng/ml-3,125ng/ml instead of 200ng/ml-1,5625ng/ml (see DFM10919).

A comparison testing of 32 individual samples was made for implementing the HV6AR00 reference. Therefore, on each of 2 assay plates 16 samples were pipetted and a serial dilution on the plate of each sample was made (1:1; 1:2, 1:4 and 1:8).

The assay plate was once measured and evaluated with the kit standard and second with the internal HV6AR00 reference. The recovery of the results from the HV6AR00 evaluated version relatively to the kit standard evaluated version was calculated.

For all tested samples the recovery was within 87%-119% and therefore within the acceptance criteria of 80%-120%.

Also, the sample serial dilution CVs [%] of the HV6AR00 evaluation are lower than the sample serial dilution CVs [%] of the kit standard evaluation. This shows that the sample dilution linearity fits much better with the internal HV6AR00 reference as with the kit standard.

### Comparison testing of 10 different lot numbers of the 75ng/ml Kit standard of the Cygnus technologies company (DFM11035)

To get an idea how much the different charges of the Cygnus kit standard vary from each other, 10 different lots of the 75ng/ml standard were tested on the same measurement plate. As reference the internal HV6AR00 reference was used (#190912/HV6AR00 – 2000ng/ml). This internal HV6AR00 was calibrated against the #300119-1 lot. After the measurement, the recoveries of the results relative to the desired values of 75ng/ml were calculated. The recoveries were from 118,9%-65,3% which demonstrates a high fluctuation from lot to lot.

F650R versus F650S kit comparison by means of F650S kit standard versus internal HV6AR00 reference comparison (DFM11525 + DFM11591)

In the next table you can see the measurement results of the F650S and F650R kit standards, when tested with F650R kit using internal HV6AR00 reference. You can see that the recoveries relative to their desired values are in the acceptance criteria for the higher concentrated standards. For the lower concentrated standards there are too high recoveries relative to the desired value (highlighted in red).



The table below shows the measurement results of different HV6AR00 and F650S kit standard concentrations, when tested with F650S kit using the kit standard respectively the HV6AR00 as reference. When tested with kit standard as reference, the 25ng/ml HV6AR00 concentration shows only 76% recovery (highlighted in red). When tested with HV6AR00 as reference the 10ng/ml F650S kit standard shows 136% recovery (highlighted in red).



A comparison of control and routine sample results between F650S kit once tested with kit standard and second tested with HV6AR00 as reference is shown in the next table. Except the “Control Sample – Low” and “Control Sample – Medium” all recoveries are within the acceptance criteria of 80%-120%. This shows that sample results are comparable between F650S kit whether using kit standard or HV6AR00 as reference.

What is also seen in the three tables of this chapter is, that the kit standard lacks linearity in the lower concentrations. This leads to undervalued results for samples with concentrations in the lower range of the reference curve. The data of the summarized experiments in this chapter show, that the supplier of the kit manufactures and sells 2ng/ml, 4ng/ml and 10ng/ml standard aliquots with much higher concentration as true value inside as indicated on analysis certificate. For this reason and to ensure acceptable sample results also in the lower measurement range a usage of the internal HV6AR00 instead of the kit standard is strongly recommended.

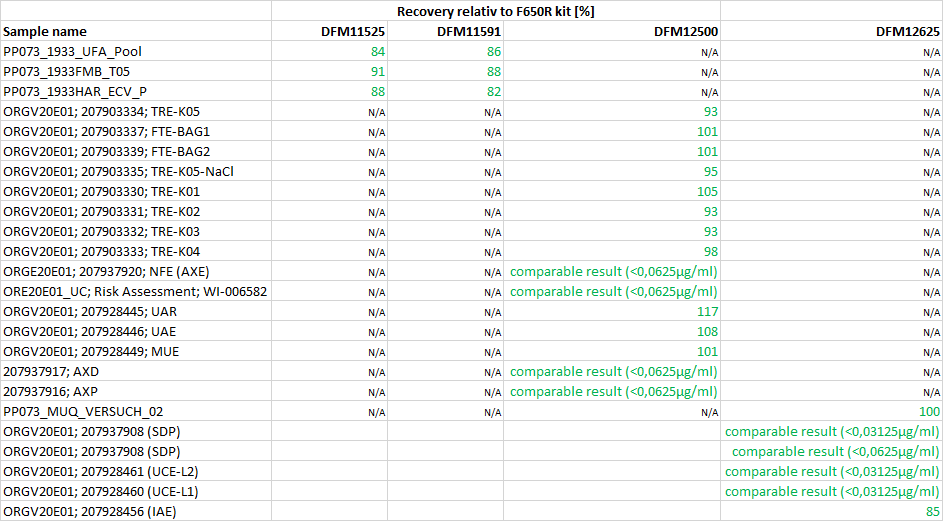
|  |  |  |  |
| --- | --- | --- | --- |
| **Name** | **Results of F650S kit tested with Kit standard (Lot.: #81119-1) [ng/ml]** | **Results of F650S kit tested with internal HV6AR00 reference [ng/ml]** | **Recovery [%]** |
| Control Sample - Low | 5,8 | 10,5 | 181 |
| Control Sample - Medium | 27,9 | 39,2 | 141 |
| Control Sample - High | 64,7 | 73 | 113 |
| E6CT\_1941\_UFA\_UDR1 | 1334500 | 1286000 | 96 |
| E6CT\_1941\_UFA\_UDR2 | 1189500 | 1233500 | 104 |
| E6CT\_1941\_UFA\_FILT | 1365500 | 1409000 | 103 |
| E6CT\_1941\_MUQ L | 1286500 | 1322500 | 103 |
| PP073\_1933\_UFA\_Pool | 149400 | 164000 | 110 |
| PP073\_1933FMB\_T05 | 71700 | 73200 | 102 |
| PP073\_1933HAR\_ECV\_P | 53050 | 53850 | 102 |
| E6CT\_1941\_UFA\_SM | 173300 | 173550 | 100 |
| E6CT\_1941\_UFA\_URA | 1947600 | 1717800 | 88 |

## GT.PA internal F650R versus F650S kit supplemental bridging testings for Hunter, TAK-686, AAV6, PPDEC, Friedreich Ataxia, DP0079, TAK-754 and TAK-748 samples (DFM11525, DFM11591, DFM11944, DFM11978, DFM12018, DFM12500 and DFM12819)

For the GT.PA internal bridging, routine samples from January 2020 until June 2020 were tested in parallel, once using F650R kit and second using F650S kit for measurement.

For some of the projects a lot more samples than for other projects were bridged just as routine samples to be available.

### Hunter (DP0073)



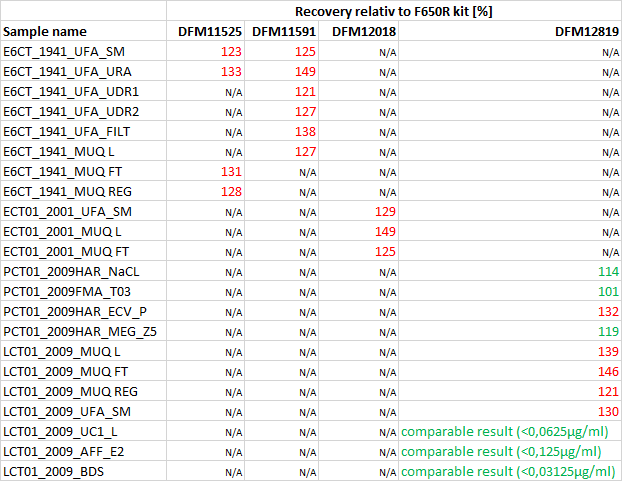
Four independent bridging tests were conducted for Hunter sample steps (DFM11525, DFM11591, DFM12500 and DFM12625). All tested Hunter samples show comparability between F650R and F650S kit (recoveries highlighted in green).

### Huntington (TAK-686)



Concerning project Huntington a shift to higher measurement results for some process steps was seen (recoveries highlighted in red).

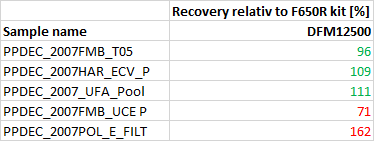
### AAV6



Four independent bridging tests were made for AAV6 samples (DFM11525, DFM11591, DFM12018 and DFM12819).

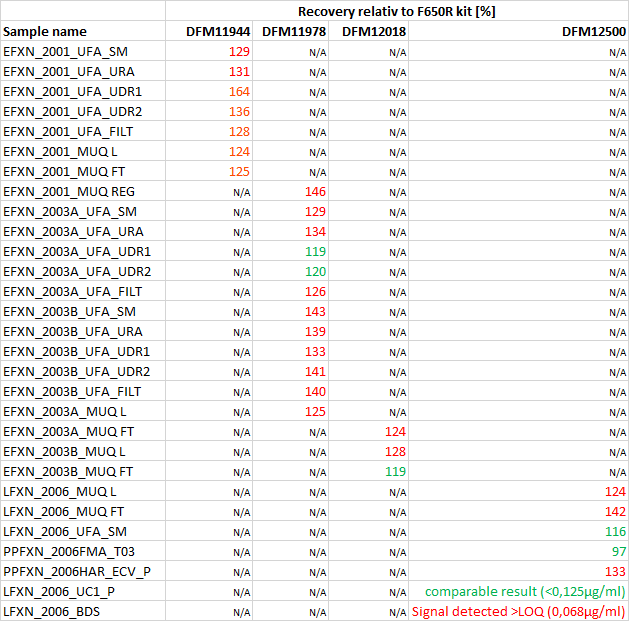
Most of the examined AAV6 samples show higher measurement results when tested with F650S kit (recoveries highlighted in red). Only for a few steps, comparability is given (highlighted in green).

### PPDEC samples



Two of the five tested PPDEC samples show either a higher or lower recovery (highlighted in red). More data would be necessary for a clear conclusion of which steps shift.

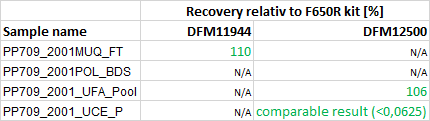
### Friedreich Ataxia



Four independent bridging tests were made for Friedreich Ataxia samples. Most of the process steps show higher measurement results when tested with F650S kit (recoveries highlighted in red). Also, four of the acceptable recoveries (highlighted in green) are from 116%-120% and therefore indicate a trend to higher measurement results for the process steps.

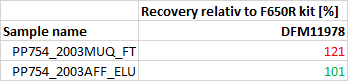
The BDS sample of Friedreich Ataxia shows no measurement signal when tested with F650R kit but when tested with F650S kit!

### FVIII 2nd Gen (DP0079)



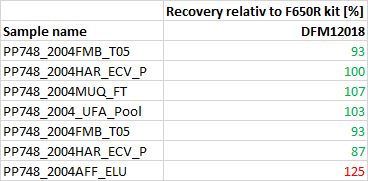
Two independent bridging tests were made with DP0079 samples. All tested samples show comparability (recoveries highlighted in green). More data would be necessary for a clear conclusion of which steps shift.

### TAK-754



The TAK-754 MUQ\_FT sample step shows a trend to higher measurement results (recovery in red). The TAK-754 AFF\_ELU step shows a comparable result (green).

### TAK-748



Except the TAK-748 AFF\_ELU step, all samples show an acceptable recovery (highlighted in green).

## Control Strategy

Blank:  
On each plate Blanks in form of the dilution buffer are measured. This ensures that any contamination in any of the reagents would be detected. As such a faulty plate can then be excluded and all samples on the plate would be repeated.

Sample predilution:

The lowest permitted dilution of samples can be found in section 6.0 of this document.

Control Sample:  
On each plate three samples with known concentrations are measured to ensure continuous results over a long period of time. Any changes or trends would be observable in the control card that is created with the values generated with each of the said control samples.  
Also any spontaneous errors that could potentially affect the samples on the plate can also be detected by the control samples and the associated control cards.

Reference Value:  
Two wavelengths are measured in each well, one at 450nm where the substrate (TMB) is absorbent and one at 620nm where nothing should absorb the light. As such the value at 620nm shows the scattering and overall loss of light and therefore the signal that is “noise” in the 450nm measurement. Additionally, it gives a reference for the “thickness” of the cuvette as the amount of liquid in each well and the resulting height acts as such.

Coefficient of Variation:  
Each sample after pre-dilution to fit into the reference curve is diluted on the plate 4 times in 1:2 dilution steps. The mean of these values is the result that is afterwards multiplied by the pre-dilution factor. Also, the Coefficient of Variation is calculated from those values to give a range for the obtained result. If this value exceeds a certain limit in regard to its result the sample can be repeated to get a smaller range.

Reference curve:

Former values until 30.10.2019 

* 200 ng/ml
* 75 ng/ml
* 25 ng/ml
* 10 ng/ml
* 4 ng/ml

Optimized curve values since 01.11.2019 with internal HV6AR00 reference (see DFM10919) 

* 200 ng/ml
* 100 ng/ml
* 50 ng/ml
* 25 ng/ml
* 12,5 ng/ml
* 6,25 ng/ml
* 3,125 ng/ml

Within 30 minutes after addition of the stop solution the plate must be measured in the plate reader at 450nm (620nm reference wavelength). The OD values from the 620nm measurement are subtracted from the 450nm measurement values (OD 450nm – OD 620nm = Delta). The OD value from the blank (=negative control) is subtracted from the Delta ODs of the standard points, controls and samples (Delta – blank = Blanked Delta = BLK delta). The BLK delta is used for all further calculations and evaluation of the results.   
The range of the measured BLK delta ODs for the highest standard point is ideally at ~2,0 OD. Values above the limit of 3,5 OD get disposed.

Using the BLK delta of the reference values and a 4-parameter fit with optimization algorithm after Levenberg-Marquardt, a calibration curve gets created. With this calculation an error in form as a R2-value is given. This error has to be below a certain point, typically <0,990. Because of its complexity, the optimization algorithm will be not further discussed in this MHF.

Respectively solving the equation after x:

y Δ OD

x concentration [%]

a minimum value (lowest possible point)

b slope (referred to point c)

c turning point of the curve

d maximum value (highest possible point)

Based on this calibration curve the concentration of the controls and samples is determined. Only values that are within the calibration curve are used for this calculation (no extrapolation is allowed).

For the calculation of final results, the measured values from the plate reader get multiplied by the predilution factor of the sample. This step is made in an exported excel file from the plate reader.

## Freeze/Thaw experiments of Anti-HEK293:HRP antibody ready-to-use solution (GN000395-019)

It was examined if the expiry date of the Anti-HEK293:HRP antibody ready-to-use solution of the F650S Kit can be extended. Therefore, several vials of the antibody solution were frozen <-60°C for several days.

A comparison testing was made with samples, once tested with the 2-8°C stored antibody solution and second with the <-60°C stored antibody solution. The recoveries of the results from the frozen antibody solution relative to the routine results were calculated.

For 10 samples comparative tested with an antibody solution frozen <-60°C for 3 days the recoveries are from 101-106% and therefore within the acceptance criteria of 80%-120%.

If the antibody solution was frozen <-60°C for 3 days AND after each day thawed at AT for 3h (3x freezing and thawing), the recoveries of the 10 samples are from 98%-106% and therefore also within the acceptance criteria.

Conclusion:

Freezing and thawing does not seem to have an impact on the measurement results.

Update: tested frozen HEK HCP antibody solution most after its expiry date.

See data generated with Hamilton in GN002569-019; 021; 023; 028; 032; 030; 035

It was found that frozen expired stocks showed a significantly lower signal for the Control (about 10% loss) which was also outside the 2s range.



Furthermore, in GN002569-083, 081 and GN002793-036. It was shown that frozen HEK HCP antibody could still be used fine for about 4 weeks post expiary date. See GN002569-083 for last valid assay.

Approximately 8 weeks after expiry date assay was invalid, see GN002793-048.

Therefore, freeze thawing does not seem to be a potential option for increasing the shelf life for more than 4 weeks.

## Adaptation of the assay conduction needed for automated testing with Hamilton pipetting robot (GN000395-025; GN000395-027)

Instead of the assay performance as quoted in the SOP, separated steps of sample incubation and antibody incubation were made.

After the measurement, the recoveries of the results relative to the routine tested samples were evaluated.

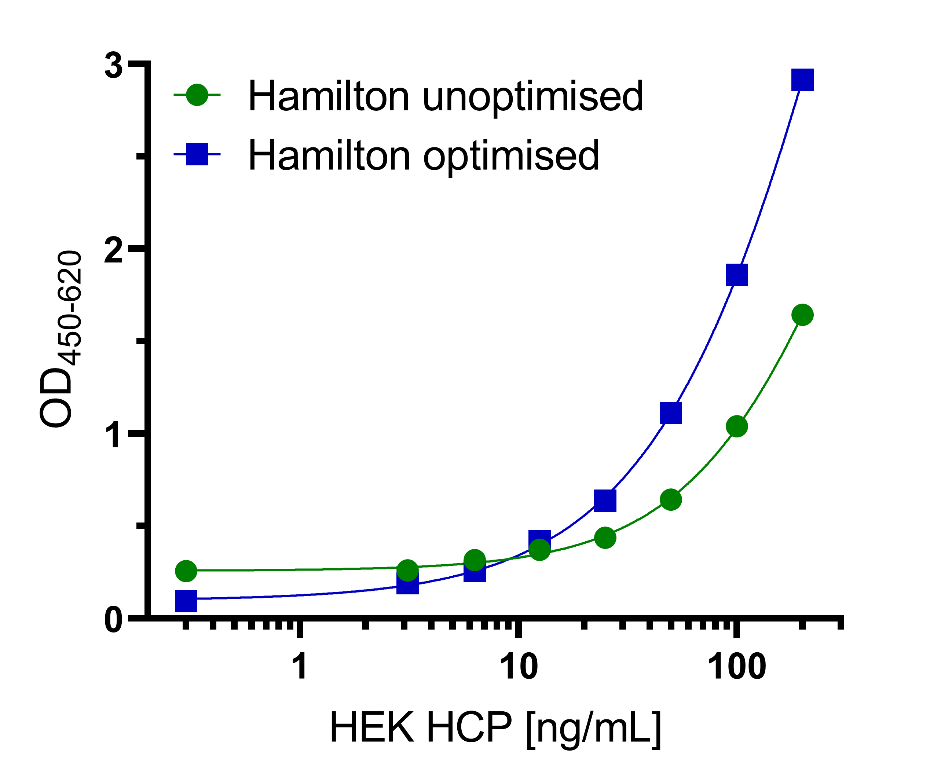
The recoveries of 23 comparative tested samples are within 105%-120% and therefore within the acceptance criteria of 80%-120%.

The recovery of 135% from the assay control is outside the acceptance criteria possibly caused by pipetting mistake.

Update: GN002569-016

Could optimize Hamilton procedure, initial wash was reduced and should be completely removed from protocol since the plate is pre-coated. Incubation times were optimized.

Adaption of the assay now acceptable on Hamilton(see slope of the standard curve at low concentration) but can still be optimized in the future.



## Substitution of Cygnus assay diluent with PBSB

Since assay diluent looks similar by inspection to PBSB and PBSB is commonly used in ELISAs, we tested the use of internally made PBSB instead of assay diluent as dilution buffer. No significant difference was found when analyzing the control sample results of 10 plates measured with cygnus assay diluent and 12 plates measured with PBSB as sample diluent.

|  |  |  |
| --- | --- | --- |
| **Sample diluent** |  |  |
| date | GN number | Control (ng/mL) |
| 4 Aug 2021 | 210729\_000395-034 | 12.46 |
| 10 Aug 2021 | 210810\_002569-003 | 11.49 |
| 19 Aug 2021 | 210810\_002569-009 | 10.42 |
| 24 Aug 2021 | 210824002569-011\_1 | 12.41 |
| 24 Aug 2021 | 210824002569-011\_2 | 13.35 |
| 31 Aug 2021 | 210824002569-012\_1 | 11.02 |
| 31 Aug 2021 | 210824002569-012\_2 | 11.15 |
| 6 Sep 2021 | 210824002569-016 | 10.10 |
| 15 Nov 2021 | 211112 002569-038 | 11.10 |
| 9 Dec 2021 | 211209 002569-048 | 9.86 |
|  |  |  |
| **PBSB** |  |  |
| date | GN number | Control (ng/mL) |
| 25 Nov 2021 | 211125 002569-040 | 13.10 |
| 1 Dec 2021 | 211125 002569-044 | 12.70 |
| 21 Dec 2021 | 211221 002569-053 | 11.90 |
| 3 Jan 2022 | 220103 002569-055 | 13.40 |
| 18 Jan 2022 | 220118 002569-061 | 10.08 |
| 27 Jan 2022 | 220127 002569-065-p1 | 13.90 |
| 27 Jan 2022 | 220127 002569-065 p2 | 13.10 |
| 15 Feb 2022 | 220215 002569-075 1 | 11.00 |
| 15 Feb 2022 | 220215 002569-075 2 | 12.00 |
| 21 Feb 2022 | 220218 002569-078 | 9.86 |
| 21 Feb 2022 | 220221 002569-079 | 13.90 |
| 25 Feb 2022 | 220225 002569-081 | 10.70 |

## Additional control K2

The control is usually being diluted 1:16, and results on average in a value of 12.6 ng/mL HEK HCP in the highest dilution on the plate. While the next dilution step (6.3 ng/mL on average) is still within the reference curve, the further two dilution steps are not.

This was set up to use the control as a limit test for HEK HCP. However, only two values are then available to calculate the control. This is quite risky since then the deviation of a single well can result in the whole plate being invalid. Therefore, the control is measured twice, once prediluted 1:16 as limit test and once 1:8 to generate additional values. All control values on the reference standard curve are then combined to calculate the control result.

## Resupply Antibody as rolled out by Cygnus in June 2022

Polyclonal Antibodies like the ones used in HEK HCP quantification are limited resource, as they are purified from pooled animal sera. By the end of 2021 Cygnus initial anti HEK HCP antibody stock was depleted. Therefore, a resupply antibody was produced by Cygnus and supplied with the Kit. Kit lots that used this resupply antibody show an **R** in their lot number. E.G.: **R**1222-322 with expiry date in Dec 2022.

It was found that this resupply antibody gives slightly different results for highly purified samples such as AFF\_ELU

And Control F653RW,

While samples like

HAR\_ECV\_P,

show similar values.

See GN003684-011

And GN003684-009

And graph

“Lot Comparison table FB”.

As of June 2022,

only resupply antibody

Lots can and will be used.

A new section in the

Control card will be established.

Initial experiment shows that so far only AFF\_ELU and Control are strongly affected. HAR, UFA, FMB, and MUQ samples are either equivalent or slightly higher than with the previous antibody Lot Kits.

Table 2: Resupply Lot to Lot comparison of Samples (GN003684-011)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | to 01 Jun 2022 | from 01 Jun 2022 |  |
| **Sample Name** | **Pre-dilution** | **Result old Lot [µg/mL]** | **Result new Lot [µg/mL]** | **Recovery of old Lot** |
| Control F653RW – 0619 | 1 | 0.0133 | 0.00822 | 62% |
| PFX07\_2208HAR\_MEG\_Z3 | 3000 | 166 | 205.2 | 124% |
| PFX07\_2208HAR\_MEG\_Z4 | 3000 | 149 | 183 | 123% |
| PFX07\_2208HAR\_ECV\_P | 3000 | 104 | 119.7 | 115% |
| PFX07\_2208\_UFA\_Pool | 20000 | 1736 | 1768 | 102% |
| PFX07\_2208\_UCE\_P | 40 | <0.125 | <0.125 | NA |
| PFX07\_2208FMB\_T03 | 500 | 17.1 | 14.8 | 87% |
| PFX07\_2208HAR\_MEG\_Z2 | 3000 | 134 | 155.7 | 116% |
| PFX07\_2208MUQ\_FT | 10000 | 787 | 844 | 107% |
| **PFX07\_2208AFF\_ELU** | 10 | 1.47 | 0.683 | 46% |
| PFB04\_2204HAR\_ECV\_P | 2000 | 151 | 192.8 | 128% |
| **PFB05\_2203AFF\_ELU** | 10 | 0.421 | 0.214 | 51% |
| PFB04\_2202 POL BDS\_pool | 10 | <0.0313 | <0.0313 | NA |
| PFB04\_2202 POL BDS\_pool Spike | target 50ng/mL | 82% recovery | 92% recovery | within 80-120% |
| EFB04\_2213\_MUQ\_FT | 10000 | 740 | 617 | 83% |
| **PFX07\_2208AFF\_ELU** | 10 | 1.47 | 0.678 | 46% |
| PFB04\_2204HAR\_ECV\_P | 2000 | 151 | 187.6 | 124% |

## Calculation of method readiness check of new HEK HCP resupply kit

The methods parameter, in alignment with the ICH-Guidelines, should be checked due to the new resupply antibody. The following data was generated in Experiments: GN003684, -014, -015, -017, -018, -020, -021.

Summary table below:

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Results** | **relevant Experiments: GN003684….** |
| Accuracy | Recovery of spiked BDS at 6 levels: 82-104% | GN0003684-017 |
| Specificity | Minimum Dilution 1:5 according to n=7 limittests | multiple: -014,-015,-017, -018, -021 |
| Recovery of limittests: 81-112% |
|  |  |
| cross-signal observed with HSA | GN003684-018 |
| Linearity | the 95% confidence interval of the slope is 0.83-0.87, thus accepted | GN0003684-017 |
| Precision – Intra Assay | measured with AFF\_ELU: CV=4.9% | GN0003684-018 |
| Precision – Inter Assay | CV of n=7 limittests 10.4% | multiple: -014,-015,-017, -018, -021 |
|  |  |
| CV-Control: 9.6% | -014,-015,-017, -018, -020, -021 |
| Quantification Limit | limittest passed for spiked BDS at 4 ng/mL | multiple: -014,-015,-017, -018, -021 |
| Range | as reference curve |  |
| Robustness | 30 min at 37°C: 92% recovery of HAR\_ECVP | GN0003684-017 |
| 2h Benchtop Recovery: 103% |
| 3 f/t-cycles Recovery: 105% |
| Lag Time: 30 min after adding Stop-Solution: 100% (Control sample) |

A limittest of n=7 spiked samples of PFB03\_2201\_POL\_BDS on different days to 4 ng/mL was conducted. The same was done for only BDS buffer. Additionally, PFB03\_2201\_POL\_BDS was also spiked to 100 ng/mL.

With the following results:

Besides a single POL\_BDS

buffer sample,

all values were within 80-120%.

Linearity was calculated as shown here: Spiked samples of POL\_BDS were plotted with their measured and theoretical values. For the last datapoint at 4 ng/mL the data of the n=7 samples of the limit test were used. ICH analytical procedure development Q14 gives an acceptance range of 0.80-1.25 for the 95% confidence interval of the slope. Thus, the acceptance range was reached, and linearity shown.



## Calculation of method readiness check of new HEK HCP resupply kit using adapted QC reference standard curve.

Since the n=7 limittest in 4.14 showed a sample with <80% recovery, and some borderline low recovery samples, the QC department wanted to focus the reference curve and set a new threshold for the limittest.

The new reference curve for the limittest consists of the following datapoints in duplicate:

128, 64, 32, 16, 12, 8, 6 ng/mL The limittest is set up for 8 ng/mL.

With this updated reference curve Method parameters are as follows:

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Results** | **relevant Experiments: GN003684….** |
| Accuracy | Recovery of spiked BDS at 4 levels: 85-102% | GN003684-026 |
| Specificity | Minimum Dilution 1:5 according to n=6 limittests | GN003684-025, -026, -028 |
| Recovery of limittests: 88-105% |
| Assume cross-signal with HSA, see above |  |
|  |
| Linearity | the 95% confidence interval of the slope is 0.99-1.07, thus accepted | GN0003684-026 |
| Precision – Intra Assay | no retesting needed | GN0003684-018 |
| Precision – Inter Assay | n=6 control samples  133 ± 6.4 ng/mL  CV = 5% | GN003684-023, -025, -026, -028, -038 |
| And GN002793-073 |
| Quantification Limit | limittest passed for spiked BDS at 8 ng/mL | -025,-026,-028 |
| Range | as reference curve |  |
| Robustness | As in table above, no retesting needed | GN0003684-017 |

An example for the used plate Layout of this reference curve is as follows:

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | Std 128ng/mL | Kontrolle 1:1 | Probe 1, 1:1 | Probe 2, 1:1 | Probe 3, 1:1 | Probe 4, 1:1 | Probe 5, 1:1 | Probe 6, 1:1 | Std 128ng/mL |  |  |  |
| B | Std 64ng/mL | Kontrolle 1:2 | Probe 1, 1:2 | Probe 2, 1:2 | Probe 3, 1:2 | Probe 4, 1:2 | Probe 5, 1:2 | Probe 6, 1:2 | Std 64ng/mL |  |  |  |
| C | Std 32ng/mL | Kontrolle 1:4 | Probe 1, 1:4 | Probe 2, 1:4 | Probe 3, 1:4 | Probe 4, 1:4 | Probe 5, 1:4 | Probe 6, 1:4 | Std 32ng/mL |  |  |  |
| D | Std 16ng/mL | Kontrolle 1:8 | Probe 1, 1:8 | Probe 2, 1:8 | Probe 3, 1:8 | Probe 4, 1:8 | Probe 5, 1:8 | Probe 6, 1:8 | Std 16ng/mL |  |  |  |
| E | Std 12ng/mL | Probe 11, 1:1 | Probe 12, 1:1 | Probe 13, 1:1 | Probe 14, 1:1 | Probe 15, 1:1 | Probe 16, 1:1 | Probe 17, 1:1 | Std 12ng/mL |  |  |  |
| F | Std 8ng/mL | Probe 11, 1:2 | Probe 12, 1:2 | Probe 13, 1:2 | Probe 14, 1:2 | Probe 15, 1:2 | Probe 16, 1:2 | Probe 17, 1:2 | Std 8ng/mL |  |  |  |
| G | Std 6ng/mL | Probe 11, 1:4 | Probe 12, 1:4 | Probe 13, 1:4 | Probe 14, 1:4 | Probe 15, 1:4 | Probe 16, 1:4 | Probe 17, 1:4 | Std 6ng/mL |  |  |  |
| H | BLK | Probe 11, 1:8 | Probe 12, 1:8 | Probe 13, 1:8 | Probe 14, 1:8 | Probe 15, 1:8 | Probe 16, 1:8 | Probe 17, 1:8 | BLK |  |  |  |

A limittest of n=6 spiked samples of PFB03\_2201\_POL\_BDS on 3 different days to 8 ng/mL was conducted. The same was done for only BDS buffer. Additionally, a POL\_BDS sample and a BDS buffer were also spiked to 100 ng/mL, once each.



With the following results:

Linearity was calculated (GN003684-026) as shown here: Spiked samples of POL\_BDS buffer were plotted with their measured and theoretical values. ICH analytical procedure development Q14 gives an acceptance range of 0.80-1.25 for the 95% confidence interval of the slope. Thus, the acceptance range was reached, and linearity shown. The same was done for BDS samples as well, and linearity was also shown, see GN003684-026 for details.



## Use of PHCP\_2302\_UFA\_Pool as new reference standard

Due to legal issues with the HV6AR00 HEK HCP standard a new reference standard PHCP\_2303\_UFA\_P was produced in-house, see DMD-225909 or legacy PRT-005534 for detailed description of the production process and DMD-226945 for risk assessment.

The relevant CoA can be found as QPL-217349 in Veeva.

In short, PHCP\_2303\_UFA\_Pool (=PHCP\_2303\_UFA\_P) was produced in a mock run of the AAV production process, where no AAV plasmids except helper plasmid were used. The material was processed up to UFA stage (10kDa ultra-filtered after harvest) and found to be representative of the production process.

In GN004325-019 the material (filled in glass vials, PHCP\_2302\_filled) was assigned the value of 6957.353 µg/mL using a bradford assay.

In GN004360-025 the filled material

was diluted (1:497) in PBSB and

aliquoted in a working stock solution

of 14.0 µg/mL (PHCP\_2302R).

In GN004360-028 it was found that

diluting that working stock 1:14 for

the first point of the reference curve

most closely resembles the

reference curve of the

old HV6AR standard.

See graph to the right.

In sum, PHCP\_2302\_UFA\_P is suitable

as reference material for the HEK HCP ELISA.

With this new reference material PHCP\_2302\_UFA\_P, Method parameters are as follows:

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Results** | **relevant Experiments:** |
| Accuracy | Recovery of spiked samples at 4 levels: 85-101% | GN004360-018,-026,-028 |
| Specificity | Recovery of limittests: 85%; 99% | GN004360-026, -028 |
| Linearity | the 95% confidence interval of the slope is 0.86-0.93, thus accepted | GN0004360-026 |
| Precision – Intra Assay | no retesting needed | GN0003684-018 |
| Precision – Inter Assay | n=6 control samples 653SW  1.12 ± 0.08 µg/mL  CV = 7% | GN004360-028, -034, -036, -039, -058 |
| Quantification Limit | limittest passed for spiked BDS buffer | GN004360-026, -028 |
| Range | as reference curve |  |
| Robustness | no retesting needed | GN0003684-017 |

## Continual Improvement

Monitor method performance that remains compliant with ATP criteria and thus analysts proactively identify and address the out-of-trend performance of the method. Update with new process and analytical technology if necessary.

# Conclusion

The method HEK 293 Host Cell Protein ELISA is fit for purpose.

Table 3: List of QFIU and FFP Matrices

| Matrix | QfIU | FFP |
| --- | --- | --- |
| BDS BAX 335 and BAX 888 | x |  |
| UAT, MUT, UBR, UBT, GT\_Mock\_FILT | x |  |
| TRT\_K03, FTT, UAR | x |  |
| UCT, SDT | x |  |
| New BDS buffer | x |  |
| Requalif. UAT, MUT, UBR, UBT (new dilutions) | x |  |
| FMB\_T01, T03, T05 for SHP648 pre-clinical prod. |  | x |
| HAR\_ECV\_P for SHP648 pre-clinical prod. |  | x |
| UFA\_UF1\_R, UFA\_Pool for SHP648 pre-clinical prod. |  | x |
| MUQ\_FT for SHP648 pre-clinical prod. |  | x |
| AFF\_ELU for SHP648 pre-clinical prod. |  | x |
| UCE\_P for SHP648 pre-clinical prod. |  | x |
| SDT\_L\_DIL for SHP648 pre-clinical prod. |  | x |
| TMAE\_E\_FILT (=BDS) for SHP648 pre-clinical prod. |  | x |
| Cell suspension samples |  | x |
| BDS in PBS + 0.001% Pluronic F68 |  | x |
| TRE-K03, FTE, UAR for SHP654 new testkit F650R | x |  |
| UAE, MUE, UBR, UBE for SHP654 new testkit F650R | x |  |
| UCE-Pool and SDP for SHP654 new testkit F650R | x |  |
| BDS for SHP654 new testkit F650R | x |  |
| MEG (mechanically disrupted): comparable to cell suspension samples processed with ultrasonic, STX: deep bed and 0.2µM filtrates of the disrupted material (like ECV\_P) – no test |  | x |
| Different buffers with MgCl2, Glycin and high molar NaCl and high Tween, sodium citrate concentrations, Histidin |  | x |
| MEC bulk - HD16 buffer |  | x |
| AFF ELU |  | x |

# List of FFP Matrices (details and references see 7.0)

## Huntington (TAK-686):

| Step | Project | FFP tested minimum sample pre-dilution | Matrix composition |
| --- | --- | --- | --- |
| MUQ\_FT | SHP686 | 2000 | 50mM Tris; 125mM NaCl; AAV9 Vector; pH= 8,5 |
| POL\_FLT (=BDS) | SHP686 | 10 | 10mM NaCitrat Dihydrat; 150mM NaCl; 0,003% Tween80; pH=7,0 ± 0,2; AAV9 Vector |
| FMB\_T03 | SHP686 | 500 | HEK (inhouse) cells in medium (1g/L Pluronic), Plasmids, PEI, AAV9 vector, transfection stop medium (CDM4HEK293), pH=7,0 |
| HAR\_MEG\_Z5 | SHP686 | 200 | Disrupted HEK cells in medium (1g/L Pluronic), Plasmids, PEI, AAV9 vector, transfection stop medium (CDM4HEK293), pH=7,0 |
| HAR\_ECV\_P | SHP686 | 500 | FE medium (1g/L Pluronic), Plasmids, PEI, AAV9 vector, CDM4HEK293, pH=7,0 |
| UFA\_Pool | SHP686 | 5000 | 50mM Tris, 125mM NaCl, AAV9 vector, pH=8,5 |
| UCE\_P | SHP686 | 20 | 20mM Tris, 137mM NaCl, sucrose (circa 52%), ~125mM MgCl2, ~50mM Natrium Acetat, AAV9 Vector |
| DIA (=BDS) | SHP686 | 10 | 50mM L-Histidine, 0,9%NaCl, 0,003% Tween80, pH=7,0, AAV9 Vector |
| AFF\_ELU | SHP686 | 40 | Mischung von Waschpuffer AAW (100mM NaAcetat, 50mM MgCl2\*6H2O, pH=5,0) und Elutionspuffer AAE (100mM NaAcetat, 1500mM MgCl2 \* 6H2O, pH=5,0) auf ca. 75mS/cm |
| E686\_2005A\_AFF\_L | DFM12819 (F650R) | 4000 | CHB - 50mM Tris, 125mM NaCl pH8,5 |
| E686\_2005A\_AFF\_E1.1 | DFM12819 (F650R) | 40 | WAB - 100mM NaAcetat, 0,1% TW80 pH6,0 |
| E686\_2005A\_AFF\_E2 | DFM12819 (F650R) | 40 | YTF - 10mM L-Histidine, 50mM Glycin, 5% Trehalose Dihydrat, 100mM NaCl, 0,005% TW80, pH7,0 |
| E686\_2005A\_AFF\_E4 | DFM12819 (F650R) | 40 | ca. 4,6mM HCl, ca. 37,8mM NaCl, Neutralisiert mit 1M Tris pH8,2 auf pH 7,95 |
|  |  |  |  |
|  |  |  |  |

## FVIII (TAK-754):

|  |  |  |  |
| --- | --- | --- | --- |
| IAT | SHP654 | 20 | 50mM Tris, 50% Ethylenglykol (EG), 750mM NaCl, AAV8 Vector, pH=8,0 |
| MUE | SHP654 | 2000 | 50mM Tris, 125mM NaCl, AAV8 Vector, pH=8,5 |
| UCE-L1 | SHP654 | 20 | 20mM Tris, 137mM NaCl, Sucrose (~53%), pH=7,4 |
| SDP | SHP654 | 20 | 20mM Tris, 137mM NaCl, Sucrose (~53%), 1:2 with 50mM Tris; pH=8,5 |
| TMAE\_NE | DFM11035 | 10 |  |
| UCE P | DFM11035 | 20 |  |

## FVIII 2nd Gen (DP0079; TAK-709):

|  |  |  |  |
| --- | --- | --- | --- |
| L709\_1924\_UFA\_SM |  | 500 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_UFA\_URA |  | 4000 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_UFA\_UDR1 |  | 4000 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_UFA\_UDR2 |  | 4000 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_UFA\_FILT |  | 4000 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| PP709\_1924FMA\_T05 |  | 1000 |  |
| PP709\_1924HAR\_ECV\_P |  | 1000 |  |
| L709\_1924\_MUQ L |  | 500 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_MUQ FT |  | 500 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_MUQ REG |  | 100 | AAV8, TAK-709 feasibility, R&D study (Ad-hoc monkey) |
| L709\_1924\_UC1\_L |  | 10 | 50mM Tris, 50% EG, 750mM NaCl pH8,0 |
| L709\_1924\_UC1\_P |  | 10 | matrix containing 45-60% sucrose in TBS |
| L709\_1924\_AFF\_E |  | 10 | 50mM Tris, 50% EG, 750mM NaCl pH8,0 |
| PP709\_2001POL\_BDS | DFM11944 | 10 |  |
| L709\_1924\_BDS | DFM10919 | 10 | ELT-Puffer |
| L709\_1924\_SDT L Dil | DFM10919 | 10 | ELT-Puffer |
|  |  |  |  |

## Hunter (DP0073):

|  |  |  |  |
| --- | --- | --- | --- |
| PP073\_1926\_UFA\_Pool | DFM10919 | 2000 |  |
| PP073\_1926\_UCE P | DFM10919; DFM11035 | 20 |  |
| PP073\_1926FMB\_T05 | DFM10919 | 500 |  |
| PP073\_1926HAR\_ECV\_P | DFM10919 | 500 |  |
| PP073\_1926MUQ\_FT | DFM10919 | 500 | 50mM Tris, 125mM NaCl pH8,5 |
| PP073\_1926AFF\_ELU | DFM10919 | 10 |  |
| PP073\_1926TMAE\_E\_FILT | DFM10919 | 10 |  |
| E073\_1936\_TMAE E | DFM11035 | 10 | ELT-Puffer |
| E073\_1936\_TMAE NE | DFM11035 | 10 | ELT-Puffer |
| E073\_1936\_UFA\_URA | DFM11035 | 2000 | F17\_Medium |
| E073\_1936\_UFA\_SM | DFM11035 | 2000 | F17\_Medium |
| E073\_1936\_UFA\_FILT | DFM10919 | 1000 | 50mM Tris, 125mM NaCl pH8,5 |
| E073\_1936\_MUQ L | DFM10919 | 700 | 50mM Tris, 125mM NaCl pH8,5 |
| E073\_1936\_MUQ FT | DFM10919 | 500 | 50mM Tris, 125mM NaCl pH8,5 |
| E073\_1936\_UFA\_UDR1 | DFM11035 | 2000 | 50mM Tris, 500mM NaCl pH8,5 |
| E073\_1936\_UFA\_UDR2 | DFM11035 | 2000 | 50mM Tris, 125mM NaCl pH8,5 |
| E073\_1936\_MUQ REG | DFM11035 | 40 | 2M NaCl |
| E073\_F17\_2010\_MUQ L | DFM13176 | 400 | F17; Xell media development Ph3 |
| E073\_F17\_2010\_MUQ FT | DFM13176 | 160 | F17; Xell media development Ph3 |
| E073\_PR1\_2010\_MUQ L | DFM13176 | 1600 | Xell 33; Xell media development Ph3 |
| E073\_PR2\_2010\_MUQ L | DFM13176 | 1600 | Xell 35; Xell media development Ph3 |
| E073\_F17\_2010\_AFF\_E | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0; Xell media development Ph3 |
| E073\_F17\_2010\_AFF\_NE | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0, Xell media development Ph3 |
| E073\_PR1\_2010\_AFF\_E | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0; Xell media development Ph3 |
| E073\_PR1\_2010\_AFF\_NE | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0; Xell media development Ph3 |
| E073\_PR2\_2010\_AFF\_E | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0; Xell media development Ph3 |
| E073\_PR2\_2010\_AFF\_NE | DFM13176 | 20 | ELB - 50mM Tris, 50% EG, 750mM NaCl pH8,0; Xell media development Ph3 |
| E073\_PR1\_2010\_MUQ L | DFM13264 | 1600 | Xell 33; Xell media development Ph3 |
| E073\_PR1\_2010\_MUQ FT | DFM13264 | 500 | Xell 33; Xell media development Ph3 |
| E073\_PR2\_2010\_MUQ L | DFM13264 | 1600 | Xell 35; Xell media development Ph3 |
| E073\_PR2\_2010\_MUQ FT | DFM13264 | 500 | Xell 35; Xell media development Ph3 |
| ORHX20004 UA-R | DFM13417 (F650S) | 200 |  |
| ORHX20004 UA | DFM13417 (F650S) | 50 |  |
| ORHX20004 MU | DFM13417 (F650S) | 50 |  |
|  |  |  |  |

## Friedreich Ataxia (EFXN samples):

|  |  |  |  |
| --- | --- | --- | --- |
| UFA\_SM | DFM11944 | 2000 | Friedreich Ataxia, AAV9\_C5, F17\_Medium |
| UFA\_URA | DFM11944 | 10000 | Friedreich Ataxia, AAV9\_C5, F17\_Medium |
| UFA\_UDR1 | DFM11944 | 5000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 500mM NaCl pH8,5 |
| UFA\_UDR2 | DFM11944 | 5000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 500mM NaCl pH8,5 |
| UFA\_FILT | DFM11944 | 5000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 500mM NaCl pH8,5 |
| MUQ L | DFM11944 | 20000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 500mM NaCl pH8,5 |
| MUQ L | DFM11978 | 20000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 125mM NaCl pH8,5 |
| MUQ FT | DFM11944 | 2000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 500mM NaCl pH8,5 |
| MUQ REG | DFM11978 | 1000 | Friedreich Ataxia, AAV9\_C5, 2M NaCl |
| AFF\_E2.1 | DFM11944 | 40 | AAW - 100mM NaAcetat, 50mM MgCl2\*6 H2O pH 5,0 |
| AFF\_E2.2 | DFM11944 | 40 | AAW - 100mM NaAcetat, 50mM MgCl2\*6 H2O pH 5,0 |
| AFF\_E3 | DFM11944 | 40 | AAW/AAE - 100mM NaAcetat, ca.250mM MgCl2\*6 H2O, 25mM Tris (mit CHB 1:2 verdünnt) |
| EMS E1 | DFM11944 | 40 | HD9 Puffer |
| EMS E2 | DFM11944 | 40 | HD9 Puffer |
| AFF\_E2 | DFM12018 | 40 | Friedreich Ataxia, AAV9\_C5, AAW/AAE - 100mM NaAcetat, ca. 620mM MgCl2\*6 H2O, pH5,0) (mit CHB 1:2 verdünnt) |
| AFF\_E2 | DFM12018 | 40 | Friedreich Ataxia, AAV9\_C5, AAW/AAE - 100mM NaAcetat, ca.763mM MgCl2\*6 H2O, pH5,0) (mit CHB 1:2 verdünnt) |
| UC1\_L |  |  | Friedreich Ataxia, AAV9\_C5, 50mM NaAcetat, ca.125mM MgCl2\*6 H2O, 25mM Tris, pH 7,6 |
| UC1\_P |  |  | Friedreich Ataxia, AAV9\_C5, matrix containing 45-60% sucrose in TBS |
| AFF\_E1 | DFM13264 | 20 | ca. 500mM L-Arginine Monohydrochlorid, ca. 400mM NaCl pH ca.5,6 |
| AFF\_NE1 | DFM13264 | 20 | ca. 1000mM L-Arginine Monohydrochlorid, ca. 800mM NaCl pH ca.5,6 |
| EFX02\_2012\_DIA | DFM13417 (F650S) | 10 | Friedreich Ataxia, AAV9\_C5, F17\_Medium |
| EFXN\_2003A\_AFF\_E2 | DFM12018 (F650S; F650R) | 40 | Friedreich Ataxia, AAV9\_C5, AAW/AAE - 100mM NaAcetat, ca. 620mM MgCl2\*6 H2O, pH5,0) (mit CHB 1:2 verdünnt) |
| EFXN\_2003B\_AFF\_E2 | DFM12018 (F650S; F650R) | 40 | Friedreich Ataxia, AAV9\_C5, AAW/AAE - 100mM NaAcetat, ca.763mM MgCl2\*6 H2O, pH5,0) (mit CHB 1:2 verdünnt) |
| EFXN\_2001\_MUQ REG | DFM11978 (F650S; F650R) | 1000 | Friedreich Ataxia, AAV9\_C5, 2M NaCl |
| EFXN\_2003A\_UFA\_URA | DFM11978 (F650S; F650R) | 20000 | Friedreich Ataxia, AAV9\_C5, F17\_Medium |
| EFXN\_2003A\_MUQ L | DFM11978 (F650S; F650R) | 20000 | Friedreich Ataxia, AAV9\_C5, 50mM Tris, 125mM NaCl pH8,5 |
|  |  |  |  |

## AAV6 (E6CT samples):

|  |  |  |  |
| --- | --- | --- | --- |
| UFA\_SM |  |  | F17\_Medium |
| UFA\_URA |  |  | F17\_Medium |
| UFA\_UDR1 |  |  | 50mM Tris, 500mM NaCl pH8,5 |
| UFA\_UDR2 |  |  | 50mM Tris, 125mM NaCl pH8,5 |
| UFA\_FILT |  |  | 50mM Tris, 125mM NaCl pH8,5 |
| MUQ L |  |  | 50mM Tris, 125mM NaCl pH8,5 |
| PCT01\_2009HAR\_NaCL | DFM12919 (F650R) | 500 |  |
| PCT01\_2009FMA\_T03 | DFM12919 (F650R) | 400 |  |
| PCT01\_2009HAR\_ECV\_P | DFM12919 (F650R) | 2000 |  |
| PCT01\_2009HAR\_MEG\_Z5 | DFM12919 (F650R) | 2000 |  |
| LCT01\_2009\_MUQ FT | DFM12919 (F650R) | 8000 | AAV6, CHQ (50mM Tris, 200 mM NaCl, pH8,5) |
| LCT01\_2009\_MUQ REG | DFM12919 (F650R) | 400 | AAV6, TWA (2M NaCl) |
| LCT01\_2009\_UFA\_SM | DFM12919 (F650R) | 2000 | AAV6, Transgene 01, F17\_Medium |
| LCT01\_2009\_UC1\_L | DFM12919 (F650R) | 20 | AAV6, Transgene 01,TBT matrix (TBS with TW80), pH 7.6 |
| LCT01\_2009\_AFF\_E2 | DFM12919 (F650R) | 40 | 15mM Tris, 0,85mM HCl, 119,13mM NaCl, 0,075%TW80 pH7,4 |
| LCT01\_2009\_BDS | DFM12919 (F650R) | 10 |  |
| PCT01\_2009HAR\_NaCL | DFM12919 (F650S) | 500 |  |
| PCT01\_2009FMA\_T03 | DFM12919 (F650S) | 400 |  |
| PCT01\_2009HAR\_ECV\_P | DFM12919 (F650S) | 2000 |  |
| PCT01\_2009HAR\_MEG\_Z5 | DFM12919 (F650S) | 4000 |  |
| LCT01\_2009\_UC1\_L | DFM12919 (F650S) | 20 | AAV6, Transgene 01,TBT matrix (TBS with TW80), pH 7.6 |
| LCT01\_2009\_AFF\_E2 | DFM12919 (F650S) | 40 | 15mM Tris, 0,85mM HCl, 119,13mM NaCl, 0,075%TW80 pH7,4 |
| LCT01\_2009\_BDS | DFM12919 (F650S) | 10 |  |
|  |  |  |  |

## DP0091 (MEC):

|  |  |  |  |
| --- | --- | --- | --- |
| GT\_MEC\_BULK18005 | DFM12625 (F650S) | 10 | Mostly empty capsids (MEC) - 10mM NaCitrat Dihydrat, 150mM NaCl, 0,003% Tween80 pH 7,0 ± 0,2 |
| GT\_MEC\_BULK18006 | DFM12625 (F650S) | 10 | Mostly empty capsids (MEC) - 10mM NaCitrat Dihydrat, 150mM NaCl, 0,003% Tween80 pH 7,0 ± 0,2 |
| GT\_MEC\_BULK18007 | DFM12625 (F650S) | 10 | Mostly empty capsids (MEC) - 10mM NaCitrat Dihydrat, 150mM NaCl, 0,003% Tween80 pH 7,0 ± 0,2 |
| GT\_MEC\_BULK18010 | DFM12625 (F650S) | 10 | Mostly empty capsids (MEC) - 10mM NaCitrat Dihydrat, 150mM NaCl, 0,003% Tween80 pH 7,0 ± 0,2 |
| GT\_MEC\_BULK18011 | DFM12625 (F650S) | 10 | Mostly empty capsids (MEC) - 10mM NaCitrat Dihydrat, 150mM NaCl, 0,003% Tween80 pH 7,0 ± 0,2 |
|  |  |  |  |

## PPDEC samples:

|  |  |  |  |
| --- | --- | --- | --- |
| PPDEC\_2007MUQ\_FT | DFM13158 (F650S); DFM12980 (F650R) | 1000 | 50 mM Tris, 125 mM NaCl, AAV8 Vector, pH 8.5 |
| PPDEC\_2007AFF\_ELU | DFM13158 (F650); DFM12980 (F650R) | 40 | 50 mM Tris, 50% Ethylenglycol, 750 mM NaCl, AAV8 Vector, pH 8.0 |

## PFB Fabry samples:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sample/Step | GN # | FFP tested minimum sample pre-dilution | Comments | Matrix composition |
| PFB04\_2204MUQ\_FT | GN002569-087 | 10000 | recovery of spiking just within 80-120% | 50mM Tris, 350mM NaCl pH8,5 |
| PFB04\_2204AFF\_ELU | GN002569-086; -087 | 10 | recovery of spiking within 80-120% | 10mM Tris, 0,5mM HCL, ~120mM NaCl,~0,03% Tween80 pH7, |
| PFB04\_2204FMB\_T03 | GN002569-086; -087 | 500 | recovery of spiking within 80-120% | Medium (1g/L Pluronic), DMEM, Plasmids, PEI, AAV9 vector, Transfection stop medium (CDM4HEK293), pH 7.0 |
| PFB04\_2204HAR\_ECV\_P | GN002569-086 | 4000 | recovery of spiking within 80-120% | As FMB\_T03 |
| PFB02\_2012\_UFA\_Pool | GN002569-087 | 20000 | recovery of spiking just within 80-120% | 50 mM Tris, 350 mM NaCl, AAV9 Vector, pH 8.5 |
| PFB04\_2204UFA\_UF1\_R | GN003684-038 | 20000 | Recovery between 80-120% | Similar to FMB\_T03 more impurities |
| PFB04\_2204HAR\_MEG\_Z4 | GN002569-086 | 4000 | F17 Medium; recovery of spiking within 80-120% | As FMB\_T03 with much more cell debris |
| PFB01\_2014\_UCE P | GN002569-093 | 20 | Recovery of 83% when diluted 1:10 and then mixed with standard solution | ~15 mM Tris, ~128 mM NaCl, ~ 52% Sucrose, ~0,015% Tween80, AAV9 Vector, pH 7,4 |
| PFB02\_2013POL\_BDS\_Pool | GN002569-093 | 10 | Recovery of 84%; standard directly spiked into sample. | 10 mM Histidine, 50 mM Glycine, 100 mM NaCl, 5% Trehalose, 0.005% Tween80, AAV9 Vector, pH 7.0 |
|  |  |  |  |  |

# Method History

Table 4: Method History

| Date | Action | Reference | Link in report |
| --- | --- | --- | --- |
| 23.08.2018 | Method transfer to Process Analytics – Gene Therapy | DFM04834 |  |
| 08.05.2018 | Preparing/aliquoting of the HEK 293 HCP Antigen control  “F653RW – 0418” | DFM04804 |  |
| 23.10.2018 | Check of dilution series instead of sample triplicates | DFM06331 |  |
| 08.02.2018 | Qualification report Analytical Development Bioanalytics Vienna | GTV0110E01 |  |
| 08.02.2018 | Qualification report Analytical Development Bioanalytics Vienna | GTV0111E01 |  |
| 08.02.2018 | Qualification report Analytical Development Bioanalytics Vienna | GTV0112E01 |  |
| 08.02.2018 | Qualification report Analytical Development Bioanalytics Vienna | GTV0113E01 |  |
| 20.09.2018 | Qualifications/FFPs details Analytical Development Bioanalytics Vienna | MHF0067E01 |  |
| 29.10.2018 | Additional conformational testing of serial dilutions instead of sample triplicates | DFM06460 |  |
| 14.11.2018 | Additional conformational testing of serial dilutions instead of sample triplicates | DFM06539 |  |
| 06.02.2019 | FFP testing for SHP686  MUQ\_FT, POL\_FLT | DFM07485 |  |
| 14.02.2019 | FFP testing for SHP686  FMB\_T03, HAR\_MEG\_Z5, HAR\_ECV\_P, UFA\_Pool, UCE\_P, DIA (BDS) | DFM07495 |  |
| 22.02.2019 | FFP testing for SHP686  AFF\_ELU | DFM07590 |  |
| 20.02.2019 | FFP testing for SHP654  UCE-L1, SDP | DFM08044 |  |
| 28.02.2019 | FFP testing for SHP654  IAT, MUE and UCE-L1 P | DFM08173 |  |
| 26.06.2019 | Preparation of a new HEK 293 HCP Antigen control ~ 333ng/ml  “F653RW – 0619” | DFM09534 |  |
| 05.08.2019 | Comparison testing TAK-748, Tak-754 and TAK-686 with cross-spiking | DFM09684 |  |
| 12.09.2019 | Internal reference preparation  HV6AR00 prediluted to 2000ng/ml and aliquoted | DFM10425 |  |
| 08.10.2019 | Comparison Testing HV6AR00 reference versus Kit standard reference | DFM10668 |  |
| 08.10.2019 | FFP testing for DP0079 samples  accuracy, repeatability, dilution linearity | DFM10668 |  |
| 30.10.2019 | Comparison Testing HV6AR00 reference versus Kit standard reference  confirmation of data from DFM10668 | DFM10919 |  |
| 30.10.2019 | FFP testing for DP0079 and DP0073 (Hunter) samples  accuracy, repeatability, dilution linearity | DFM10919 |  |
| 13.11.2019 | FFP testing of TAK-754 step TMAE\_NE + UCE\_P; FFP testing of Hunter samples (DP0073);  Comparison of 10 different lots of the 200ng/ml Cygnus technologies company kit standard | DFM11035 |  |
| 14.01.2020 | F650R versus F650S kit comparison and F650S kit standard versus internal HV6AR00 reference comparison; GT.PA internal bridging testings of AAV6 and Hunter samples | DFM11525 |  |
| 21.01.2020 | F650R versus F650S kit comparison and F650S kit standard versus internal HV6AR00 reference comparison; GT.PA internal bridging testings of AAV6 and Hunter samples | DFM11591 |  |
| 17.02.2020 | FFP testing for steps of Friedreich Ataxia; GT.PA internal bridging testings of Friedreich Ataxia and DP0079 samples | DFM11944 |  |
| 19.02.2020 | FFP testing for EFXN samples (Friedreich Ataxia); GT.PA internal bridging testings of Friedreich Ataxia and TAK-754 samples | DFM11978 |  |
| 28.02.2020 | FFP testing of EFXN samples (Friedreich Ataxia); GT.PA internal bridging testings of Friedreich Ataxia, AAV6 and TAK-748 samples | DFM12018 |  |
| 20.04.2020 | FFP testing for GT\_MEC\_BULK samples; GT.PA internal bridging testings of PPDEC and Hunter samples | DFM12625 |  |
| 09.04.2020 | GT.PA internal bridging testings of PPDEC, TAK-686, Friedreich Ataxia and Hunter samples | DFM12500 |  |
| 20.05.2020 | FFP testing for steps of projects AAV6 and Friedreich Ataxia; GT.PA internal bridging testings of AAV6 samples | DFM12819 |  |
| 23.06.2020 | FFP testing on F650S kit for PPDEC samples (project: dedicated empty capsids batch for data generation and analytical supply) of step MUQ\_FT and AFF\_ELU | DFM13158 |  |
| 25.06.2020 | FFP testing on F650R kit for PPDEC samples (project: dedicated empty capsids batch for data generation and analytical supply) of step MUQ\_FT and AFF\_ELU | DFM12980 |  |
| 25.06.2020 | Hunter Xell media development FFP testing | DFM13176 |  |
| 15.07.2020 | Hunter and Friedreich Ataxia FFP testing | DFM13264 |  |
| 16.07.2020 | Friedreich Ataxia and Hunter LIMS sample FFP testing | DFM13417 |  |
| 26.08.2020 | FFP testings for PFBxx samples | DFM13800 |  |
| 23.09.2020 | FFP testings for PFBxx samples | DFM13922 |  |
| 27.01.2022 | Freeze/Thaw experiments of Anti-HEK293:HRP antibody ready-to-use solution | GN000395-019 | 4.7 |
| 15.04.2022 | Adaptation of the assay condition needed for automated testing with Hamilton pipetting robot | GN000395-025; GN000395-027 | 4.8 |
| 31.03.2022 | Added data for change of cygnus assay diluent to PBSB as diluent. Added use of additional control K2.  Added spiking experiments for PFB samples and resulting minimal dilution. | GN002569-086;  GN002569-087; | 4.9  4.10 |
| 08.04.2022 | Update on Freeze/Thaw experiments added to the section | GN002793-048  GN002569-083  GN002569-081 | 4.7 |
| 08.04.2022 | Update on Hamilton pipetting robot adaption added to section | GN0025690-16 | 4.8 |
| 10.05.2022 | Showing equivalence between PBSB and Cygnus dilution buffer and use of additional K2 sample | Multiple, see section for data | 4.9  4.10 |
| 10.06.2022 | Antibody change to new resupply kit by Cygnus, and comparison table to old kit | GN003684-009  GN003684-011 | 4.11 |
| 20.06.2022 | Method parameters of new resupply kit by Cygnus | Multiple, see section | 4.12 |
| 01.08.2022 | Method parameters of new resupply kit by Cygnus using QC reference curve.  Also revision of document | Multiple, see section | 4.13 |
| 01.07.2023 | Use of PHCP\_2302\_UFA\_P reference standard | Multiple, see section | 4.14 |

# Referenced Documents

## VV-00944864 Analytical Method Lifecycle (TO SOP-1981)

## VV-00940890, Method Development, Qualification and Validation

## ICH Q2(R1) Validation of Analytical Procedures

## SOP-051247: HEK 293 Host Cell Protein ELISA; PA-GT

## Method History File (Analytical Development Bioanalytics Vienna): MHF0067E01

## DMD-225909 Production of an in-house standard for the HEK293 host cell protein assay

## DMD-226945 HEK HCP ELISA - Risk Assessment for implementation of a new Reference Standard

## QPL-217349 Certificate of Analysis HEK293-Reference material

# Version History

| Rev. # | Justfication/Summary of Changes |
| --- | --- |
| 1 | Creation of this document and continuous addition of new data |
| 2 | Revision for transfer to QC department |
| 3 | Revision for use of reference PHCP\_2302 in QC department |

Link to living document: <https://mytakeda.sharepoint.com/:w:/r/sites/GeneTherapyAnalytics2/_layouts/15/Doc.aspx?sourcedoc=%7B7E3E0B8E-241F-44F6-8CAD-4A7672FDE422%7D&file=DMD-212109_HEK293-HostCellProtein_ELISA.docx&action=default&mobileredirect=true>