



## *EScalate<sup>®</sup> Plasma Protein Binding Assay Service*

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### Assay Principle

The *EScalate* assay comprises a microtiter plate-based two-dimensional dilution matrix of blood plasma and HSA-coated beads plus a set of appropriate controls. The impact of increasing plasma concentration on the binding equilibrium of a test compound to HSA-coated beads is determined experimentally. Samples are subsequently quantified by LC-MS. From the acquired data set, the free fraction ( $f_u$ ) of the test compound in plasma can be calculated. Results can be determined for any species for which plasma is available. For details please refer to the published application notes <sup>1,2</sup>.

### Test compounds and plasma

At least 5 mg of each test compound or 100 µl of a 10 mM stock solution is required. The total molecular weight has to be known for MS analysis. Availability of an appropriate internal standard (ideally isotope labeled, 1 mg or 20 µl of a 10 mM stock solution) will improve data quality. Per assay approximately 0.6 - 1 ml blood plasma are required. Plasma may be supplied by the study sponsor or will be provided by 3B Pharmaceuticals. It is strongly recommended to use only one type of anticoagulant in comparative studies.

### Deliverables

3B Pharmaceuticals is providing a report in pdf-format for each series of PPB measurements containing the determined free fraction values and a description of the relevant assay and analysis parameters.

### Process and turnaround time

For each compound both sample preparation and the analytical method have to be developed. Samples are quantified by LC-MS. The typical turnaround time from receipt of the test items at 3B Pharmaceuticals' facilities in Berlin to results delivery is four to six weeks.

### Service contract

3B Pharmaceuticals has a sample master service agreement in place outlining the terms and conditions of the *EScalate* service offering.

### References

1. 3B Pharmaceuticals GmbH, 3B Pharmaceuticals Application note 1: Determination of the plasma protein binding of Liraglutide using the *EScalate* Assay. **2017**.
2. 3B Pharmaceuticals GmbH, 3B Pharmaceuticals Application note 2: Determination of the plasma protein binding of Carbamazepine, Desipramine, Pyrimethamine and Warfarin using the *EScalate* assay. **2017**.

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