

Aug 03, 2025

In-vitro plasma protein binding

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5o866g1b/v1>

Nick Lynch^{1,2}

¹Curlew Research; ²ASAP Discovery Consortium

ASAP Discovery



Nick Lynch

Curlew Research, ASAP Discovery Consortium

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN  ACCESS



DOI: <https://dx.doi.org/10.17504/protocols.io.8epv5o866g1b/v1>

Protocol Citation: Nick Lynch 2025. In-vitro plasma protein binding. protocols.io
<https://dx.doi.org/10.17504/protocols.io.8epv5o866g1b/v1>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: May 25, 2025

Last Modified: August 03, 2025

Protocol Integer ID: 218905

Keywords: ADME, DMPK, drug discovery, plasma protein, drug interaction, protein binding, accurate prediction of human pharmacokinetic parameter, human pharmacokinetic parameter, plasma protein, quantitative measurements essential for comprehensive drug development program, unbound drug fraction, protein level, binding study, drug development, equilibrium dialysis, administered drug, influences drug efficacy, protein, understanding drug behaviour, comprehensive drug development program, bound compound

Disclaimer

The protocol content here is for informational purposes only and does not constitute legal, medical, clinical, or safety advice, or otherwise; content added to protocols.io is not peer reviewed and may not have undergone a formal approval of any kind. Information presented in this protocol should not substitute for independent professional judgement, advice, diagnosis, or treatment. Any action you take or refrain from taking, using or relying upon the information presented here is strictly at your own risk. You agree that neither the Company nor any of the authors, contributors, administrators, or anyone else associated with protocols.io, can be held

Abstract

In vitro plasma protein binding studies in various animal models (Human, rat, mouse, dog, fetal calf serum) are crucial for understanding drug behaviour and predicting human responses. These studies help determine how a drug interacts with plasma proteins, influencing its distribution, metabolism, and elimination.

Protein binding significantly influences drug efficacy and safety profiles. Highly bound compounds (>90%) exhibit greater sensitivity to changes in protein levels due to disease states, age, or co-administered drugs. These data support regulatory submissions and inform clinical trial design by enabling accurate prediction of human pharmacokinetic parameters from preclinical models.

The assay employs equilibrium dialysis, ultrafiltration, or ultracentrifugation methods to separate bound and unbound drug fractions, providing quantitative measurements essential for comprehensive drug development programs.

Safety warnings

- ⚠ Always wear appropriate PPE for this protocol
Refer to Material Safety Data Sheets for additional safety and handling information.

Summary

1 Plasma protein binding assay is performed in 48-well RED device

This protocol can be used with the following protein plasma:

- Rat
- Mouse
- Fetal calf serum
- Dog
- Human

Sample Preparation

2 The compounds are incubated at 5 μ M in a shaking incubator at 37°C

3 The plasma is diluted to 50% with pH 7.4 phosphate buffer and warmed to 37°C for 10min, test compound is then added to achieve 5 μ M

Assay

4 500 μ L of dialysis buffer is added to one side of the chamber of the RED device insert housed within the heated Teflon block, and the incubation is initiated by the addition of 300 μ L of the test compound protein solution to the opposing chamber. The final solvent concentrations are 0.95% Methanol and 0.05% DMSO (99% plasma).

5 The samples are incubated for 4 hours to allow equilibrium to be reached

6 Equal volumes from the buffer and the plasma chambers (50 μ L) are transferred into separate wells of a deep well plate

6.1 50 μ L of 50% blank plasma are added to the buffer samples, and 50 μ L of buffer are added to the 50% plasma samples, followed by mixing (this ensures matrix matching of samples from both the free and bound fractions)

6.2 300 μ L of ice cold acetonitrile containing internal standard are added to precipitate the protein

7 The quenched samples are then centrifuged to precipitate the protein.



Analytical

- 8 The supernatants are analyzed by LC-MS/MS using generic analytical methods to quantify the concentration of test compound in each chamber.

Data Analysis

- 9 The fraction unbound is calculated by dividing the concentration in the buffer chamber by the concentration in the plasma chamber.

For each test compound injection,

response ratio = Test peak area / Internal standard peak area

From the standard curve, the response ratio is converted to concentration of test compound.

The binding value of test compound bound is calculated from,

Fraction Unbound = Concentration buffer chamber / Concentration plasma chamber

% Free = Fraction Unbound X 100

% Bound = 100% - % Free

The % Recovery is calculated via:

% Recovery = (Concentration buffer chamber + Concentration plasma chamber) * 100 /
Total quantity incubated

Protocol references

[https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05d023a60f6a6c23c3f6d_Concept%20Life%20Sciences_LE_Protein_Binding%20\(1\).pdf](https://cdn.prod.website-files.com/659db4dde7f13f395f49157c/65f05d023a60f6a6c23c3f6d_Concept%20Life%20Sciences_LE_Protein_Binding%20(1).pdf)

Acknowledgements

Grateful to Concept Life Sciences for supplying the original protocol summary