IAM Chromatography

Immobilized Artificial Membrane (IAM) technology is an innovative approach to chromatography in which the chromatographic surface emulates the lipid environment of the cell membrane.[1,2]

HPLC Separation Tools for Membrane Protein Purification and Drug Membrane Permeability Prediction

Phosphatidylcholine (PC) is the major phospholipid found in cell membranes. IAM chromatography phases prepared from PC analogs closely mimic the surface of a biological cell membrane. Consequently, IAM phases display a high affinity for membrane proteins and are useful in membrane protein purification and in the study of drug-membrane interactions. The IAM surface is formed by covalently bonding the membrane-forming phospholipids to silica. Several different types of IAM columns are used for various applications:

Membrane Protein Purification

IAM.PC
IAM.PC.MG

Drug Discovery

IAM.PC.DD2

- Drug membrane permeability prediction
- Hydrophobic in nature

IAM Fast-Screen Mini Columns

- High throughput estimation of drug permeability

Membrane Protein Purification

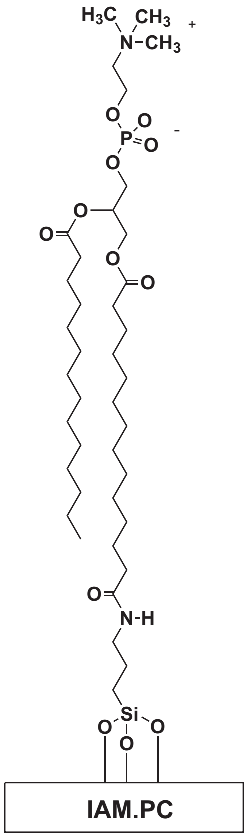


Figure 1. The Phosphatidylcholine is covalently bound to propylamine groups, which are in turn bound to silica. Because the bulky PC groups limit access to the unbonded amine groups, these may or may not affect the separation of a given protein.

IAM.PC

The IAM.PC phase, developed by Dr. Charles Pidgeon of Purdue University, was the first in a line of IAM phases to be manufactured by Regis. Use of this phase has simplified the inherent difficulties of protein isolation and purification,[3-9] allowing for rapid purification of membrane proteins while maintaining biological activity. The IAM.PC phase is an important tool for the pharmaceutical industry and academia alike.

The first IAM stationary phase was based on the prevalent membrane lipid, phosphatidylcholine (PC), and consists of monolayers of amphiphilic phospholipids covalently bonded to aminopropyl silica particles through a terminal amide linkage. As a result, the bulky phosphatidylcholine groups shield many of the amine binding sites on the silica surface, preventing amine interaction with the protein molecules.

The membrane nature of the IAM phase imparts surface characteristics which are useful in the chromatography of membrane proteins. These include: high protein loading, increased protein recovery, recovery of functional activity, and selectivity for membrane proteins.

Large membrane proteins can interact with any combination of polar headgroup, hydrophobic chain, or inner amine groups. The subsurface has been shown to interact with certain solutes, and may or may not contribute to the separation of a given biomolecule. The residual amines can be left unaltered on the subsurface or deactivated through an endcapping procedure, which results in increased stability of the bonded phase. The methyl glycolate endcapping, for example, converts residual amines to neutral amides and introduces a hydroxyl group (IAM.PC.MG).

IAM.PC Applications

Numerous applications have been developed using IAM.PC columns:

- Purification of Cytochrome P450
- Isolation of membrane proteins
- Prediction of solute transport across human skin
- Prediction of amino acid transport across the blood-brain barrier
- Binding of solutes to liposome membranes
- Immobilization of Trypsin and α-chymotrypsin for the determination of their inhibitor and substrate activity

For additional information on IAM.PC applications please contact Regis' technical support staff.

Product	Particle Size	Column Dimensions	Catalog #
IAM.PC	10 μm, 300Å	3 cm x 4.6 mm i.d.	770007
IAM.PC	10 μm, 300Å	15 cm x 4.6 mm i.d.	770001
IAM.PC.MG	10 μm, 300Å	3 cm x 4.6 mm i.d.	772007
IAM.PC.MG	10 μm, 300Å	15 cm x 4.6 mm i.d.	772001
IAM.PC Guard Kit*	10 μm, 300Å	1 cm x 3.0 mm i.d	771001
IAM.PC Guard Cartridges**	10 μm, 300Å	1 cm x 3.0 mm i.d	774001
IAM.PC.MG Guard Kit*	10 μm, 300Å	1 cm x 3.0 mm i.d	773001
IAM.PC.MG Guard Cartridges**	10 μm, 300Å	1 cm x 3.0 mm i.d	775001

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

Drug Discovery-Predicting Drug Membrane Permeability

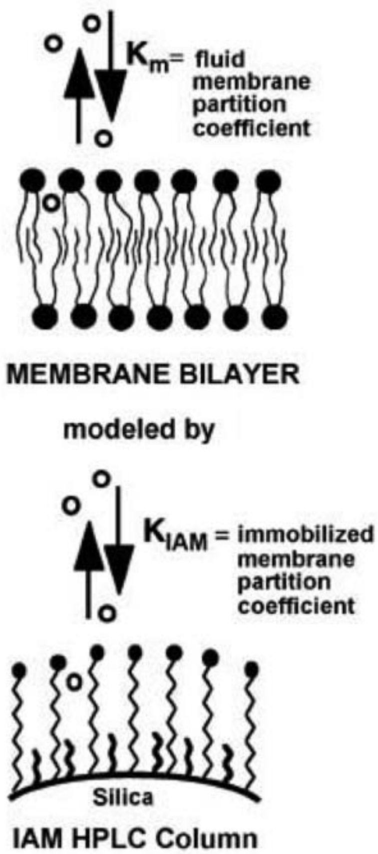


Figure 2. Fluid membrane bilayer can be modeled by IAM column.

IAM.PC.DD2
IAM Fast-Screen Mini Column

IAM chromatography has recently gained acceptance among drug discovery chemists for estimating the membrane permeability of small molecule drugs.

Figure 2 illustrates that the interaction between membrane bilayer and drug can be modeled by the IAM column/drug system.

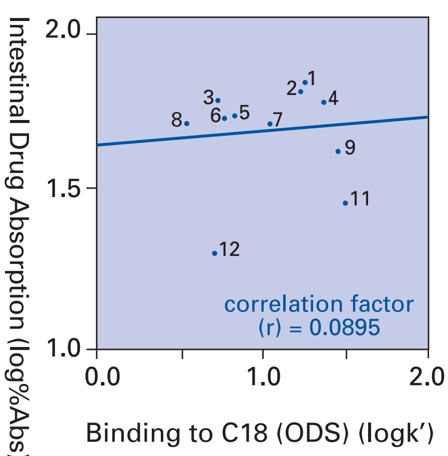
K_{IAM}, the equilibrium constant describing the relative concentrations of drug in the membrane and in the external fluid, is analogous to the k'_{IAM}.

This IAM technique provides superior correlation with experimentally determined drug permeability when compared to other chromatographic methods. ODS silica, for example, retains analytes solely on the basis of hydrophobicity. IAM more closely mimics the interaction of analytes with biological membranes, where a combination of hydrophobic, ion pairing, and hydrogen bonding interactions are possible. This combination of interactions measured by the IAM column is known as phospholipophilicity.

These advances have led to the development of several new IAM phases used for predicting drug membrane permeability:

- IAM.PC.DD2
- IAM Fast-Screen Mini Column

ODS Exhibits Poor Correlation with Intestinal Drug Absorption	
Column:	C18 (ODS)
Mobile Phase:	3 cm x 4.6 mm i.d.
Flow Rate:	1.0 mL/min
Load:	10 µL
Detection:	UV 220 nm



1. *m*-Nitroaniline
2. *p*-Nitroaniline
3. Salicylic acid
4. *p*-Toluidine
5. Aniline
6. *m*-Nitrobenzoic acid
7. Phenol
8. Benzoic acid
9. Acetanilide
10. Antipyrine
11. Theophylline
12. Acetylsalicylic acid

Figure 3. Drug partitioning into ODS does not correlate with intestnal drug absorption.

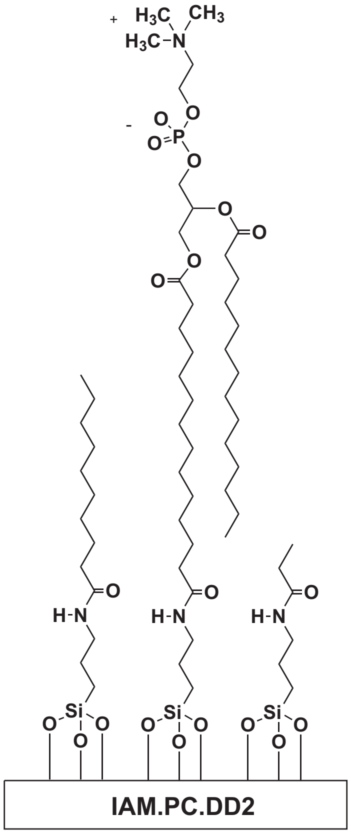


Figure 4. IAM.PC.DD2 is used to predict drug membrane permeability.

Intestinal Drug Permeability

The retention factors measured on reversed phase C18 (ODS) columns (a commonly used model to determine drug partitioning) show extremely poor correlation with intestinal drug absorption (figure 3). For this group of compounds, hydrophobicity alone, as measured by the reversed-phase C18 column, is a poor predictor of drug absorption. Since IAM.PC Drug Discovery columns measure both hydrophilic and hydrophobic interactions between drugs and membranes, the IAM.PC Drug Discovery Column is better suited to the prediction of intestinal drug absorption.

Like the first generation IAM.PC.DD material, the IAM.PC.DD2 is used to predict drug membrane permeability. The ester bonding of the DD2 packing offers more hydrophobicity than the first generation DD phase. This material is a diacylated or double chain ester PC ligand and is endcapped with C10/C3 alkyl chains as illustrated in figure 4.

Column Advantages

The IAM.PC.DD2 material offers the following advantages:

- Hydrophobic nature
- Greater stability
- Excellent correlation to traditional methods

Hydrophobic Nature

The IAM.PC.DD2 offers more hydrophobicity than the first generation IAM.PC.DD material. This hydrophobic nature allows for longer retention times to compounds not well retained on the IAM.PC.DD material.

Greater Stability

Another distinct advantage of the IAM.PC.DD2 material is its ability to tolerate mobile phases between pH's 7.0 to 7.5, thus resulting in longer column life under these conditions.

Excellent Correlation to Traditional Methods

The traditional means of predicting membrane permeability include the use of Caco-2 cell line cultures, intestinal tissue or liposome assays. These methods are laborious and costly to perform.

IAM.PC.DD2

Sample	% Absorption of Inverted Rat Intestine	(k') IAM.PC.DD2
m-nitroaniline	77	10.838
p-nitroaniline	68	16.086
salicylic acid	60	6.963
p-toluidine	59	4.546
aniline	54	2.069
m-nitrobenzoic acid	53	4.403
phenol	51	6.544
benzoic acid	51	2.088
acetanilide	42	5.096
antipyrine	32	3.350
theophylline	29	1.478
acetylsalcylic acid	20	0.931
r (correlation factor)*		**0.8025**

*r is calculated by plotting log k' vs. log % absorption ofinverted rat intestine.

Table 1. Correlating Drug Partitioning into IAM with rat intestinal drug absorption.

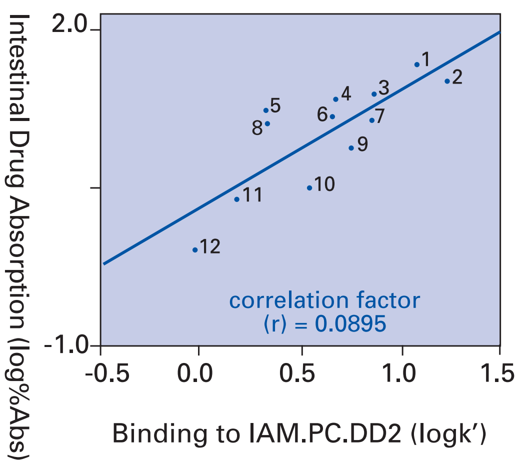
Intestinal Tissue Correlation

Measuring drug permeability in the intestinal tissue, where absorption is occurring, is physiologically more relevant than measuring drug permeability in Caco-2 cells. Figure 5 and table 1 illustrate that drug absorption in this inverted rat intestinal tissue model correlates with drug retention factors k'_{IAM} measured on the IAM.PC.DD2 column.

Product	Particle Size	Column Dimensions	Catalog #
IAM.PC.DD2	10 µm, 300Å	3 cm x 4.6 mm i.d.	774010
IAM.PC.DD2	10 µm, 300Å	10 cm x 4.6 mm i.d.	774011
IAM.PC.DD2	10 µm, 300Å	15 cm x 4.6 mm i.d.	774014
IAM.PC.DD2 Guard Kit*	10 µm, 300Å	1 cm x 3.0 mm i.d	774012
IAM.PC.DD2 Guard Cartridges**	10 µm, 300Å	1 cm x 3.0 mm i.d	774013

* Includes 1 holder and 2 guard cartridges
** Includes 3 guard cartridges

IAM Correlates with Intestinal Drug Absorption	
Column:	IAM.PC.DD2
	10 cm x 4.6 mm i.d.
Mobile Phase:	0.01 M DPBS Buffer, pH 5.4
Flow Rate:	1.0 mL/min
Load:	10 µL
Detection:	UV 220 nm



1. *m*-Nitroaniline
2. *p*-Nitroaniline
3. Salicylic acid
4. *p*-Toluidine
5. Aniline
6. *m*-Nitrobenzoic acid
7. Phenol
8. Benzoic acid
9. Acetanilide
10. Antipyrine
11. Theophylline
12. Acetylsalicylic acid

Figure 5. IAM.PC.DD2 columns measure drug absorption in inverted rat intestinal tissue.

IAM Fast Screen Mini Column

Method	Number of Compounds Evaluated	Correlation (r) with IAM Fast-Screen Mini Column
Partitioning into liposomes	23	0.831
Intestinal drug permeability	12	0.839
Caco-2 cell permeability	8	0.909

Table 2. Comparing k'IAM data with other methods for estimating permeability.

Compound	% Absorption of Inverted Rat Intestine	IAM Fast-Screen Mini Column Retention Time (Sec)	
m-nitroaniline	77	133.1	15.29
p-nitroaniline	68	177.9	21.84
salicylic acid	60	93.8	9.54
p-toluidine	59	79.7	7.48
aniline	54	52.1	3.45
m-nitrobenzoic acid	53	68.1	5.79
phenol	51	94.6	9.66
benzoic acid	51	43.7	2.22
acetanilide	42	76.2	6.97
antipyrine	32	51.8	3.40
theophylline	29	39.3	1.58
acetylsalcylic acid	20	36.1	1.11
r (correlation factor)*			**0.8385**

Table 3. Correlating drug partitioning into IAM with rat intestinal drug absorption.

* r is calculated by plotting log k' vs. log % absorption of inverted rat intestine.

Packed with the Ester PC Ligand phase, IAM Fast-Screen Mini columns are a rapid and economically viable screening method for the high throughput estimation of drug permeability. Their benefits include excellent reproducibility, short analysis time and low cost. This can be of great use in characterizing large libraries of compounds. The structure of the ^{ester}IAM.PC.C10/C3 packing, selected for the Fast-Screen Mini Column, is shown in figure 6. This PC analog demonstrates superiority in retention times and stability–essential features for short columns and mass drug screening. The IAM.PC Fast-Screen Mini Column, 1 cm in length by 3.0 mm in internal diameter, was specifically designed by Regis for rapid estimation of drug permeability in high throughput screening programs. When connected to an HPLC system with an autosampler, a single column can be used in the analysis of hundreds of samples per day with highly reproducible results. The 1 cm Fast-Screen Mini Column is offered not as a separation tool, but rather as a tool for characterizing the chromatographic retention factor (k') of individual analytes. The measured k' of analytes on this column can be used to estimate a value for drug permeability.

Column Advantages

Regis Technologies' 1 cm Fast-Screen Mini Column for Drug Discovery provides the following advantages:

- Excellent correlation to traditional methods
- Rapid indication of drug absorption
- High sample throughput
- Highly reproducible results
- Durability
- Cost effectiveness
- Ability to establish absorption zones for high throughput screening IAM Fast-Screen Mini Column Structure

Excellent Correlation To Traditional Methods

The traditional means of predicting permeability include use of Caco-2 cell line cultures, intestinal tissue, or liposome assays. These are laborious and costly to perform. Data obtained from the IAM Fast-Screen Mini Column correlate well to data obtained from traditional assays. This is summarized in table 2.

Chromatographic Conditions:	
Column:	IAM Fast-Screen Mini Column 1 cm x 3.0 mm i.d.
Mobile Phase:	Dulbecco's Phosphate Buffered Saline, pH 5.4
Flow Rate:	0.3 mL/min
Load:	10 µL
Detection:	UV 254 nm, 0.1 AUFS

IAM Fast Screen Mini Column

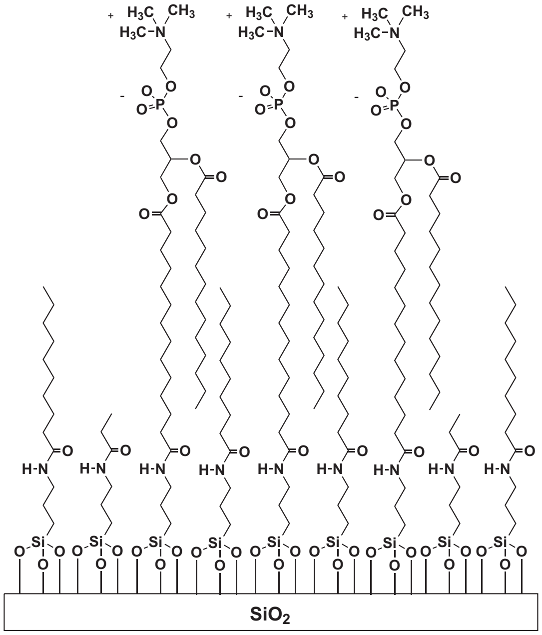


Figure 6. IAM.PC Fast-Screen Mini Column provides rapid estimation of drug permeability in high thoughput screening programs.

IAM Fast-Screen Correlates with Drug Permeability in Caco-2 Cells	
Column:	IAM Fast-Screen Mini Column 1 cm x 3.0 mm i.d.
Mobile Phase:	0.01 M DPBS Buffer, pH 7.4
Flow Rate:	0.5 mL/min
Load:	10 µL
Detection:	UV 220 nm

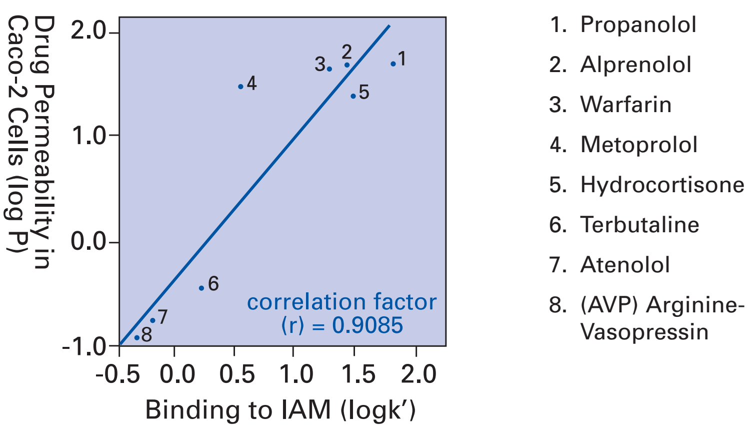


Figure 7. Correlating drug partitioning into IAM with intestinal drug permeability (log P) through Caco-2 cells.

Intestinal Tissue Correlation

Table 3 shows that drug permeability predicted by Inverted Rat Intestines correlates well to drug retention factors, k'_{IAM} measured on the IAM Fast-Screen Mini Columns. Note the short retention times.

Caco-2 Cell Correlation

Figure 7 illustrates that drug permeability predicted by Caco-2 cells correlates well to k'_{IAM} measured on the IAM Fast-Screen Mini Columns.

Rapid Indication of Drug Absorption

IAM Chromatography is a more rapid alternative to other methods. In a recent study completed by Regis, k'_{IAMs} of 12 compounds were compared with absorption data obtained in situ using rat intestines. Retention times for the compounds tested were between 20 and 180 seconds, while retention factors correlated well to the intestinal absorption data.

High Sample Throughput

IAM chromatography is of increasing importance in combinatorial chemistry, where it is used to provide an initial estimate of a drug candidates' membrane permeability. Hundreds of samples can be injected into a single Fast-Screen Mini Column using an automated HPLC system. Recently a group of 12 test analytes was evaluated in 10 runs over the course of eight hours. Total run time for the 12 test analytes was only 42 minutes.

Highly Reproducible Results

The measured values for k'_{IAM} show excellent reproducibility, both from run to run and from day to day (figure 8).

Durability

IAM Fast-Screen Mini Columns are extremely durable. Correlation factors, r, for the original k', and k' after 5000 column volumes were identical.

Cost Effectiveness

Because the IAM Fast-Screen Mini Column is inexpensive, has a very short analysis time, and provides drug permeability estimates for hundreds of drug candidates in a fraction of the time of conventional methods, the IAM Fast-Screen Mini Column becomes the economical alternative for high throughput screening.

Ability to Establish Permeability Zones for High Throughput Screening

Permeability zones can be determined for different analytes when performing large-scale drug absorption screening. Thus, rapid IAM analyses can characterize a drug as having low, medium, or high membrane permeability (figure 9).

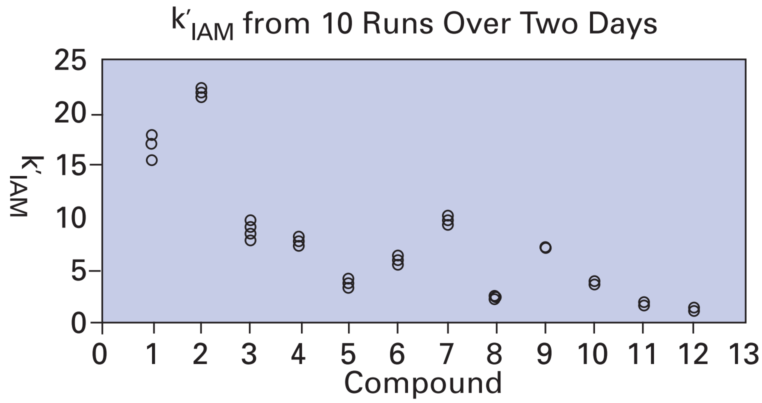


Figure 8. Highly reproducible k'_{IAM} from 10 runs over a two-day period.

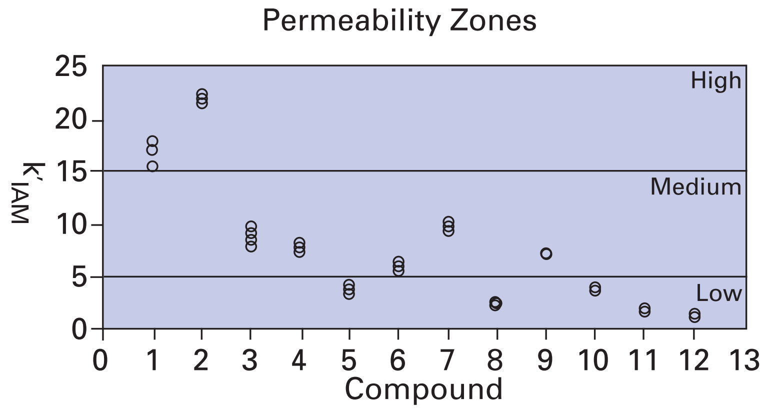


Figure 9. Permeability zones established large-scale drug absorption screening.

IAM Fast Screen Mini Column

Regis Technologies manufactures the IAM Fast-Screen Mini Column on-site in its manufacturing facility. This column, as well as all of our other products, must adhere to rigorous manufacturing and quality control specifications before release.

Regis' technical support staff, with years of chromatography experience, is available to answer any questions regarding the new IAM Fast-Screen Mini Column.

Product	Particle Size	Column Dimensions	Catalog #
IAM Fast-Screen Mini Column Kit • 2 columns, 1 cm x 3 mm • 1 cartridge holder • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775014
IAM Fast-Screen Mini Columns, Pkg. of 6 • 6 columns, 1cm x 3 mm • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775015
IAM Fast-Screen Mini Columns, Pkg. of 12 • 12 columns, 1 cm x 3 mm • Care and use booklet	10 µm, 300Å	1 cm x 3.0 mm i.d	775016

IAM References

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