

AGP kit

TRANSIL AGP Binding Kit

CATALOG #
AAA60730

PRODUCT SYNONYMS

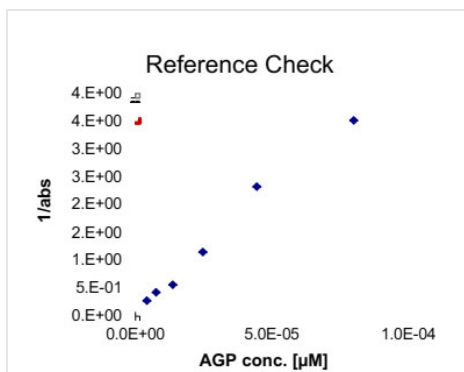
AGP; TRANSIL AGP Binding Kit; AGP kit

PRODUCT SPECIFICATIONS

Preparation and Storage

Store at -20 degree C

IMAGES



Standard Curve (Sample)

Related Product Information for AGP kit	Background: It is commonly accepted that only the unbound fraction (fu) of a drug can penetrate cell membranes and exert its pharmacological effect or become available for elimination. Many drugs, especially lipophilic compounds, bind to circulating plasma proteins, such as human serum albumin (HSA), alpha1-acid glycoprotein (AGP), globulins, and lipoproteins. Among these proteins, HSA and AGP are the most important for the reduction of the exposure to xenobiotics because of their ability to bind a large array of structurally unrelated drugs in distinct binding sites (Kremer et al., 1988; Peters, 1996). Human serum albumin (HSA) is the most abundant protein in blood plasma. It is synthesized in the liver and concentrations in healthy subjects normally range between 35 and 60 g/L with an average of 42 g/L (Peters, 1996). HSA comprises 60% of the total plasma proteins. Its main physiological function is to bind and carry endogenous anions, with longchain fatty acids. Two high affinity binding sites have been proposed in subdomains IIA (also known as Sudlow's site I or warfarin site) and IIIA (also known as Site II or benzodiazepine site) of HSA. These comprise highly elongated hydrophobic pockets with charged lysine and arginine residues near the surface that interact with polar ligand parts. In contrast to AGP, HSA blood levels are much more stable. Hyperalbuminemia (HSA > 55 g/L) is seldom seen in the absence of dehydration whereas hypoalbuminemia is the more common condition resulting from malnutrition or liver disease with serum albumin levels dropping to 20-23 g/L (Peters, 1996). Human AGP, also called orosomucoid, is a small acute-phase glycoprotein (about 40 kD), synthesized mostly by hepatocytes and is present in the plasma of healthy subjects at concentrations that range from 0.36 to 1.46 g/L with a mean of 0.77 g/L (Blain et al., 1985), accounting for about 1% to 3% of total plasma protein. AGP exhibits a sequence homology of 75% with immunoglobulins (Schmid et al., 1973) and functions as a carrier protein for basic and neutral lipophilic endogenous compounds such as steroid hormones or xenobiotics (Kremer et al., 1988). AGP levels can vary considerably in response to disease with values up to 2.8 g/L observed in patients with viral or bacterial infections (Voulgari et al., 1982) or 10-fold differences (0.3-3 g/L) being found in cancer patients (Jackson et al., 1982). Moreover, AGP is expressed at lower levels in the fetus (0.15 g/L) than the mother (0.5 g/L), resulting in a higher free fraction of drugs, e.g. propranolol and lidocaine, in neonatal blood than in maternal blood (Wood M & Wood AJ, 1981). It has been shown that plasma levels of AGP inversely correlate with the fu of alprenolol (Piafsky & Borga, 1977), docetaxel (Loos et al., 2003), disopyramide (Bredesen & Kierulf, 1986), imipramine (Piafsky & Borga, 1977), and propranolol (Piafsky et al., 1978). Significant differences in the fu of disopyramide between population of Black (Johnson & Livingston, 1997), Chinese (Zhou et al., 1990) and White backgrounds suggest differences in AGP levels. In a recent clinical study, AGP could be identified as an independent predictor for a clinical endpoint, i.e. survival in patients with non-small cell lung cancer treated with docetaxel (Bruno et al., 2003). Given the clinical relevance of fluctuation in the fu altering drug disposition (Summerfield et al., 2006), active transport (Bow et al., 2006), drug-drug interactions (Christensen et al., 2006) and drug efficacy, especially in the field of chemotherapy of infectious diseases (Boffito et al., 2002; Zhang et al., 1999) there is an increased understanding that binding to plasma proteins should be investigated in the early stages of the drug discovery process. Unfortunately, existing methods for determination of plasma protein binding have low capacity and/or high cost and lack standard evaluation procedures (for a review, see Boffito et al., 2003). Conventional methods to determine plasma protein binding comprise equilibrium dialysis, ultrafiltration, and ultracentrifugation (Sebille, 1990), ideally using plasma samples of pooled healthy donors, usually with no control over HSA and AGP levels. High throughput methods have been described for plasma equilibrium dialysis combined with liquid chromatography/ mass spectrometry (LC-MS/MS) analytics (Wan & Rehngren, 2006). Notably, the resolution of these methods is limited, particularly when examining drugs that are highly bound to plasma proteins. These conventional methods require highly sensitive analytical techniques that exhibit a linear range of more than two orders of magnitude such as LC-MS/MS or the use of radiolabeled compounds to resolve plasma protein binding of compounds with fu-values smaller than 0.01. Given the above constraints for the determination of plasma protein binding, the TRANSIL HSA and TRANSIL AGP Assay kits were developed and validated employing a novel method that overcomes these limitations and addresses the problem of varying HSA and AGP levels. To overcome the analytical limitations, when examining drugs that are highly bound to plasma proteins, the TRANSIL HSA and TRANSIL AGP Binding assays allow KD-values to be determined by titrating different subphysiological concentrations of HSA and supraphysiological concentrations of AGP against a constant drug concentration. Principle of the Assay: The principle of the TRANSIL AGP Binding Kit is to assess the affinity of test compounds to human alpha1-acid glycoprotein (AGP). AGP binding is determined by incubating a fixed concentration of drug candidate with varying concentrations of alpha1-acid glycoprotein immobilized on silica beads. A total of 8 wells of a tube unit are used to determine the plasma protein binding for each compound (Figure 1). Six wells contain AGP covered silica beads, while two serve as references to account for non-specific binding and contain buffer only. Using the spreadsheet and algorithms supplied with kit, the affinity to albumin is calculated from remaining free compound concentration in the supernatant of each well. Any of the available detection systems, such as LC-MS/MS, scintillation counting, etc. can be used for quantification, as long as it can quantify uM concentrations in volumes of 50 ul or less.
Product Categories/Family for AGP kit	Sovicell ADME Assays

For Research Use Only. Not for use in diagnostic or therapeutic procedures. Small volumes of product may become entrapped in the seal of the vial during shipment and storage; if necessary, briefly centrifuge the vial on a tabletop centrifuge to dislodge any liquid in the cap.

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Online datasheet: <https://stage.aaabiotech.com/kits/agp/60730>