Human Serum Albumin (HSA)

Albumin is a main protein of blood plasma carrying multiple functions. It is synthesized by liver and secreted into the bloodstream as a 67 kDa single-chain protein. Its' tertiary structure is sustained by 17 disulfide bonds. Albumin concentration in plasma is about 40 mg/ml that is more than a half of the total plasma protein concentration. Albumin regulates filtration and absorption of fluid across capillary walls since up to 80% of blood osmotic pressure is determined by albumin. It also binds and transports in blood a lot of substances including fatty acids, steroid hormones, bilirubin, tryptophan, calcium ions as well as drugs such as penicillin, aspirin, dicumarin, or sulfonamides.

Albumin concentration in blood decreases usually either because of liver diseases leading to a derangement of protein synthesis or because of its loss with urine in renal diseases. Albumin excretion with urine normally is less than 20 μg/min. An increase in albumin concentration in urine is called albuminuria. The urinary excretion of albumin within 20 – 200 μg/min (30 – 300 mg per day) is defined as microalbuminuria; the albumin excretion greater than 200 μg/min is called clinical albuminuria. The latter usually reflects overt nephropathy (glomerular affection) and could be detected by a sulfosalicylic acid method or a dipstick method.

Microalbuminuria has been considered to be an early marker of nephropathy and reflects microvascular damage over the blood circulating system. Microalbuminuria is a risk marker of cardiovascular complications in patients with diabetes mellitus (1). It predicts renal disease, left ventricular dysfunction, stroke, myocardial ischemia and infarction (2). In addition, microalbuminuria has been found in primary hypertensive patients, in patients with atrial fibrillation, carotid and femoral atherosclerosis, hepatitis C infection (3).

Presence of albumin in urine could be detected by several methods, but immunochemical approach is the most sensitive and specific. Immunoassays, utilizing albumin-specific antibodies could be used for albumin quantifications not only in urine, but also in other biological fluids. To select antibodies which are most suitable for microalbuminuria diagnosis, Hytest's specialists have tested several dozens of albumin-specific monoclonal antibodies and several hundreds of two-site MAb combinations. Sensitive, precise, and rapid assay for reliable quantitative albumin immunodetection in human urine was our goal, and we succeeded in gaining it.

MONOCLONAL ANTIBODIES SPECIFIC TO HUMAN SERUM ALBUMIN

More than 30 hybridomas producing albumin-specific MAbs were generated after immunization of Balb/c mice with human serum albumin. Monoclonal antibodies presented here are albumin specific and do not have cross-reactivity with other tested human proteins.

Albumin detection in Western blotting

All antibodies presented here could be used for the detection of human serum albumin in Western blotting after gel electrophoresis under reducing and non-reducing conditions (Fig. 1).

MAbs for albumin extraction from human serum (plasma)

Anti-albumin MAbs could be used for the preparation of immunosorbents to deplete serum of albumin that is a necessary stage of proteomic studies of blood proteins.

Development of a sandwich immunoassay

All Hytest's albumin-specific MAbs were tested in sandwich immunoassay as capture and detection antibodies to select the best two-site MAbs combination for the development of quantitative sandwich immunoassay. The selected MAbs combinations are presented in Table 1.

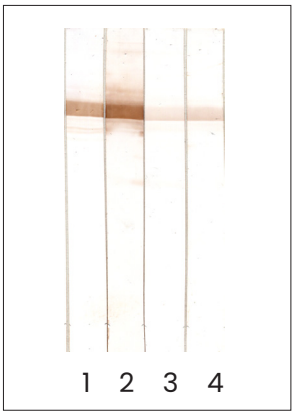

Figure 1.
Albumin staining by five albumin-specific MAbs in Western blotting after electrophoretic separation of human plasma proteins in reducicng conditions.
Lane 1: MAb 6B11
Lane 2: MAb HSA11
Lane 3: MAb 14E7
Lane 4: MAb 15C7

Table 1.
Antibody pair recommendations.

Capture	Detection
HSA20	14E7
15C7cc	1A9
15C7cc	6B11
HSA11	15C7cc
1A9	15C7cc

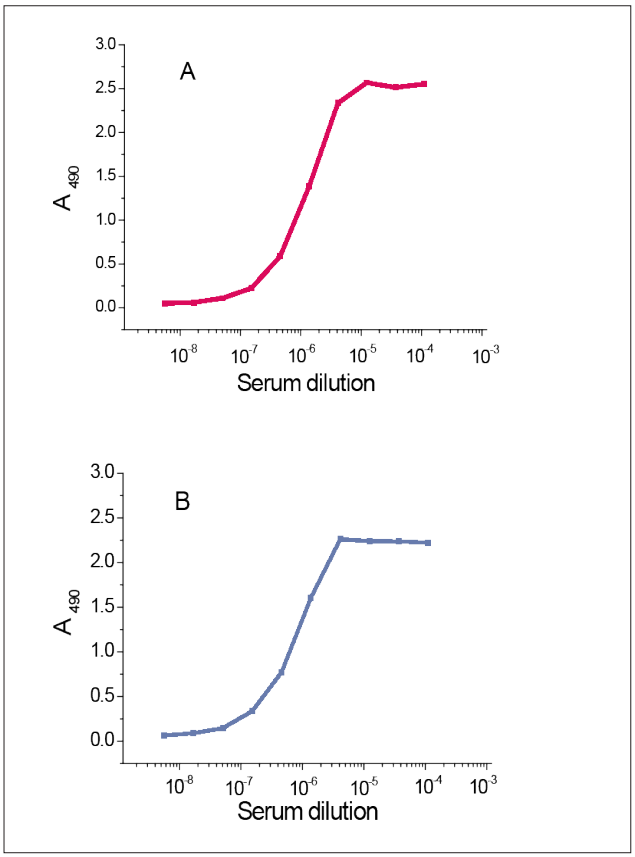

Figure 2.
Titration of human serum in two albumin sandwich immunoassays (capture - detection).
A: 15C7 – 1A9 and B: HSA20 – 14E7.

REFERENCES

1. **Bennett PH et al.** Screening and management of microalbuminuria in patients with diabetes mellitus: recommendations to the Scientific Advisory Board of the National Kidney Foundation from an ad hoc committee of the Council on Diabetes Mellitus of the National Kidney Foundation. Am J Kidney Dis. 1995, 25(1), pp.107-112.
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ORDERING INFORMATION

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Human serum albumin (HSA)	4T24	1C8	IgG1	EIA, WB
		1A9	IgG2a	EIA, WB
		6B11	IgG2a	EIA, WB
		14E7	IgG2b	EIA, WB
		HSA11	IgG1	EIA, WB
		HSA20	IgG1	EIA, WB
	4T24cc	15C7cc	IgG2b	*In vitro*, EIA, WB

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced *in vivo*. MAbs produced *in vitro* are expected to have similar performance.

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