SBA Clonotyping System: AP Kit User Guide

Cat. No: OKSB00002
Lot. No: KF0330

For Research Use Only

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Description

The SBA Clonotyping System-AP kit is designed for the isotyping of mouse monoclonal antibodies. It contains 2.5 mg of capture antibody and 1.0 mL of AP conjugated anti-mouse Ig, mouse IgA, mouse IgG_1, mouse IgG_{2a}, mouse IgG_{2b}, mouse IgG_3, mouse IgM, mouse , mouse , and pNPP substrate. The kit may also be utilized for quantitative studies of mouse immunoglobulins in samples such as serum, supernatant, and ascites when used in conjunction with the Mouse Immunoglobulin Panel (Aviva Cat. No. OKSB00001).

Applications

ELISA – Quality tested [1-29]

ELISPOT – Reported in literature [16,27,30,31]

Kit Components

Goat Anti-Mouse Ig, Human ads-UNLB
Goat Anti-Mouse Ig, Human ads-AP
Goat Anti-Mouse IgA-AP
Goat Anti-Mouse IgG_1, Human ads-AP
Goat Anti-Mouse IgG_{2a}, Human ads-AP
Goat Anti-Mouse IgG_{2b}, Human ads-AP
Goat Anti-Mouse IgG_3, Human ads-AP
Goat Anti-Mouse IgM, Human ads-AP
Goat Anti-Mouse Kappa-AP
Goat Anti-Mouse Lambda-AP
pNPP Substrate Tablets

Handling and Storage

The purified (UNLB) antibody is supplied as 2.5 mg purified immunoglobulin in 1.0 mL of borate buffered saline, pH 8.2. *No preservatives or amine-containing buffer salts added*. Store at 2-8°C.

The alkaline phosphatase (AP) conjugates are supplied as 1.0 mL of stock solution in 50 mM Tris/1 mM $MgCl_2$/50% glycerol, pH 8.0, containing NaN_3 as preservative. Store at 2-8°C or long-term at -20°C.

The pNPP substrate tablets are supplied as 20 x 5 mg. Recommended storage is at -20 C. Protect from light.

Reagents are stable for the period shown on the label if stored as directed.

Warning

Some reagents contain sodium azide.

Suggested Isotyping Protocol

Dilute capture antibody to a concentration of 5 - 10 g/mL in borate buffered saline (BBS), pH 8.2 or phosphate buffered saline (PBS), pH 7.4; add 0.1 mL to each well of the ELISA plate; alternatively, the antigen used for immunization may be used as the coating reagent

Cover plate with a lid or plastic wrap and incubate in a humidified atmosphere at 2-8 C for a minimum of 12 hours

Empty wells, wash 3X with BBS (or PBS) containing 0.05% Tween®, empty wells, and fill wells with BBS (or PBS) containing 1% bovine serum albumin (BBS/BSA)

Allow antibody-coated plate to stand at room temperature for a minimum of 1 hour to block free binding sites on the plate Empty plate and wash 3X

Add 0.1 mL of hybridoma supernatant to each well, cover plate, and incubate for 1 hour at room temperature with gentle shaking or overnight at 2-8 C

Empty plate and wash 3X

Dilute AP-labeled detection antibody(ies) 1:250 – 1:500 in BBS/BSA, add 0.1 mL conjugate(s) to appropriate wells of the plate, cover plate, and incubate for 1 hour at room temperature with gentle shaking or overnight at 2-8 C

Empty the plate and wash 5X

Prepare substrate buffer - To 400 mL of double glass-distilled water, add 24.5 mg $MgCl_2 \cdot 6H_2O$ and 48 mL diethanolamine; adjust pH to 9.8 with 5N HCl and make up to 500 mL with distilled water

Prepare a 1 mg/mL substrate solution (e.g., one 5 mg tablet + 5 mL substrate buffer) and add 0.1 mL to each well of the plate Read optical density of each well at 405 nm after substrate addition

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References

1. Bergen HR 3rd, Losman MJ, O'Connor T, Zacharias W, Larson JE, Accavitti MA, et al. Specificity of monoclonal anti-Z-DNA antibodies from unimmunized MRL/Mp-lpr/lpr mice. J Immunol. 1987;139:743-8. (ELISA - Isotyping)
2. Hrdličková R, Nehyba J, Humphries EH. v-rel induces expression of three avian immunoregulatory surface receptors more efficiently than c-rel. J Virol. 1994;68:308-19. (ELISA - Isotyping)
3. Hong JS, Engler JA. Domains required for assembly of adenovirus type 2 fiber trimers. J Virol. 1996;70:7071-8. (ELISA - Isotyping)
4. Beier RC, Ripley LH, Young CR, Kaiser CM. Production, characterization, and cross-reactivity studies of monoclonal antibodies against the coccidiostat nicarbazin. J Agric Food Chem. 2001;49:4542-52. (ELISA - Isotyping)
5. Itoh T, Yoshida M, Chiba T, Kita T, Wakatsuki Y. A coordinated cytotoxic effect of IFN-γ and cross-reactive antibodies in the pathogenesis of Helicobacter pylori gastritis. Helicobacter. 2003;8:268-78. (ELISA - Isotyping)
6. Beelen R, Boyd B, Garavello JC, Pavanelli GC, Ainsworth AJ. Generation, characterisation and applicability of a monoclonal antibody to hybrid surubim catfish Pseudoplatystoma corruscans (Agassiz) × Pseudoplatystoma fasciatum (Linnaeus) immunoglobulin. Comp Clin Path. 2004;12:191-8. (ELISA - Isotyping)
7. Moore-Lai D, Rowland E. Discovery and characterization of an antibody, anti-egressin, that is able to inhibit Trypanosoma cruzi egress in vitro. J Parasitol. 2004;90:524-30. (ELISA - Isotyping)
8. Beier RC, Creemer LC, Ziprin RL, Nisbet DJ. Production and characterization of monoclonal antibodies against the antibiotic tilmicosin. J Agric Food Chem. 2005;53:9679-88. (ELISA - Isotyping)
9. Liu J, Liu B, Cao Z, Inoue S, Morita K, Tian K, et al. Characterization and application of monoclonal antibodies specific to West Nile virus envelope protein. J Virol Methods. 2008;154:20-6. (ELISA - Isotyping)
10. Marsh MB, Rice CD. Development, characterization, and technical applications of a fish lysozyme-specific monoclonal antibody (mAb M24-2). Comp Immunol Microbiol Infect Dis. 2010;33:e15-23. (ELISA - Isotyping)
11. Schreeder DM, Cannon JP, Wu J, Li R, Shakhmatov MA, Davis RS. Cutting edge: FcR-like 6 is an MHC class II receptor. J Immunol. 2010;185:23-7. (ELISA - Isotyping)
12. Merrell K, Wells S, Henderson A, Gorman J, Alt F, Stall A, et al. The absence of the transcription activator TFE3 impairs activation of B cells in vivo. Mol Cell Biol. 1997;17:3335-44. (ELISA - Serum & Supernatant)
13. Test ST, Mitsuyoshi J, Connolly CC, Lucas AH. Increased immunogenicity and induction of class switching by conjugation of complement C3d to pneumococcal serotype 14 capsular polysaccharide. Infect Immun. 2001;69:3031-40 (ELISA - Serum)
14. Schmidt CS, Liu J, Zhang T, Song HY, Sandusky G, Mintze K, et al. Enhanced B cell expansion, survival, and humoral responses by targeting death receptor 6. J Exp Med. 2003;197:51-62. (ELISA - Serum)
15. Test ST, Mitsuyoshi JK, Hu Y. Depletion of complement has distinct effects on the primary and secondary antibody responses to a conjugate of pneumococcal serotype 14 capsular polysaccharide and a T-cell-dependent protein carrier. Infect Immun. 2005;73:277-86. (ELISA - Serum)
16. Shimoda M, Li T, Pihkala JP, Koni PA. Role of MHC class II on memory B cells in post-germinal center B cell homeostasis and memory response. J Immunol. 2006;176:2122-33. (ELISA - Serum, ELISPOT)
17. Shankar M, Nixon JC, Maier S, Workman J, Farris AD, Webb CF. Anti-nuclear antibody production and autoimmunity in transgenic mice that overexpress the transcription factor Bright. J Immunol. 2007;178:2996-3006. (ELISA - Serum)
18. Nixon JC, Ferrell S, Miner C, Oldham AL, Hochgeschwender U, Webb CF. Transgenic mice expressing dominant-negative bright exhibit defects in B1 B cells. J Immunol. 2008;181:6913-22. (ELISA - Serum)
19. Bynoté KK, Hackenberg JM, Korach KS, Lubahn DB, Lane PH, Gould KA. Estrogen receptor-α deficiency attenuates autoimmune disease in (NZB x NZW)F_1 mice. Genes Immun. 2008;9:137-52. (ELISA - Serum)
20. Finetti F, Pellegrini M, Ulivieri C, Savino MT, Paccagnini E, Ginanneschi C, et al. The proapoptotic and antimitogenic protein p66SHC acts as a negative regulator of lymphocyte activation and autoimmunity. Blood. 2008;111:5017-27. (ELISA - Serum)
21. Weant AE, Michalek RD, Khan IU, Holbrook BC, Willingham MC, Grayson JM. Apoptosis regulators Bim and Fas function concurrently to control autoimmunity and $CD8^+$ T cell contraction. Immunity. 2008;28:218-30. (ELISA - Serum)
22. Savino MT, Ortensi B, Ferro M, Ulivieri C, Fanigliulo D, Paccagnini E, et al. Rai acts as a negative regulator of autoimmunity by inhibiting antigen receptor signaling and lymphocyte activation. J Immunol. 2009;182:301-8. (ELISA - Serum)
23. Hinman RM, Nichols WA, Diaz TM, Gallardo TD, Castrillon DH, Satterthwaite AB. Foxo3-/- mice demonstrate reduced numbers of pre-B and recirculating B cells but normal splenic B cell sub-population distribution. Int Immunol. 2009;21:831-42. (ELISA - Serum)
24. Cash H, Relle M, Menke J, Brochhausen C, Jones SA, Topley N, et al. Interleukin 6 (IL-6) deficiency delays lupus nephritis in MRL-Fas^{lpr} mice: the IL-6 pathway as a new therapeutic target in treatment of autoimmune kidney disease in systemic lupus erythematosus. J Rheumatol. 2010;37:60-70. (ELISA - Serum)
25. Ohnmacht C, Schwartz C, Panzer M, Schiedewitz I, Naumann R, Voehringer D. Basophils orchestrate chronic allergic dermatitis and protective immunity against helminths. Immunity. 2010;33:364-74. (ELISA - Serum)
26. Webb CF, Bryant J, Popowski M, Allred L, Kim D, Harriss J, et al. The ARID family transcription factor bright is required for both hematopoietic stem cell and B lineage development. Mol Cell Biol. 2011;31:1041-53. (ELISA - Serum)
27. Anginot A, Espeli M, Chasson L, Mancini SJ, Schiff C. Galectin 1 modulates plasma cell homeostasis and regulates the humoral immune response. J Immunol. 2013;190:5526-33. (ELISA - Serum & Supernatant, ELISPOT)
28. Yoachim SD, Nuxoll JS, Bynoté KK, Gould KA. Estrogen receptor alpha signaling promotes Sle1-induced loss of tolerance and immune cell activation and is responsible for sex bias in B6.Sle1 congenic mice. Clin Immunol. 2015;158:153-66. (ELISA - Serum)
29. Guillaume V, Contamin H, Loth P, Grosjean I, Courbot MC, Deubel V, et al. Antibody prophylaxis and therapy against Nipah virus infection in hamsters. J Virol. 2006;80:1972-8. (ELISA - Ascites)
30. Bolduc A, Long E, Stapler D, Cascalho M, Tsubata T, Koni PA, et al. Constitutive CD40L expression on B cells prematurely terminates germinal center response and leads to augmented plasma cell production in T cell areas. J Immunol. 2010;185:220-30. (ELISPOT)
31. Park KS, Bayles I, Szlachta-McGinn A, Paul J, Boiko J, Santos P, et al. Transcription elongation factor ELL2 drives Ig secretory-specific mRNA production and the unfolded protein response. J Immunol. 2014;193:4663-74. (ELISPOT)

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