

SDS-Gel Electrophoresis and blotting: Kidney protein extracts were mixed with 2X standard Laemmli **reducing** buffer, heated for 5 min at 90oC. Approx **20 µg total proteins** were run on **4-20%-reducing SDS-mini gels** at 200 V for approx. 45 min. Multi-colored **high range mol. Wt markers** (ADI Cat # HMWM-11) were loaded on each gel: **A** (Aprotinin, Blue, 6.5K); **B** (Lactalbumin, purple, 14.2K); **C** (Soybean trypsin inhibitor, green, 20.1); **D** (Carbonic anhydrase, orange, 29K); **E** (Ovalbumin, yellow, 45K); **F** (bovine serum albumin, pink, 66K); **G** (beta-galactosidase, turquoise, 116K), and **H** (muscle myosin, blue, 205K). The proteins were transferred to PVDF using mini-transblot cells. Homogeneity of protein transfer in all 8 lanes was verified using water soluble Stain-ALL (ADI Cat # SALL-500) for 5 min. Protein lanes were identified and marked 1-8. Membranes were washed in PBS to remove the dye. **Multi-colored mol. Wt standards (Lane 1) have been marked A-H on the blot.** TOP and BOTTOM of gel are indicated by two arrows on the blot.

Blocking: After destaining, membranes were blocked with 1:10 diluted PBS/milk-based buffer (ADI Cat# 80062) and air-dried.

Form, Storage, and Recommended Usage: Blots are provided pre-blocked and in ready-to-use forms. Store unused blots at 4oC in a sealed bag. ReadyBlot should be handled with care, as blotting membrane is quite brittle. These blots should be used within 3-4 months. It is recommended to soak the blot first in 100% methanol until the blot is wet followed by washing with PBS twice to remove any residual methanol before incubating with antibodies. It is also recommended to keep the blot in PBS+azide if blots are to be re-used (stripped) to study with another antibody.

This blot will be most useful for proteins that are relatively abundant in whole kidney tissue. Very low abundant proteins that require the use of enriched cell membranes or nuclear fractions may be poorly represented in whole tissue blots.

Ordering Information

Adult **Mouse** ReadyBlot **Kidney** Protein Explorer

Cat # MKWB-61; (Mouse: Swiss Webster, ~10 wks old, mixed gender)

Adult **Rat** ReadyBlot **Kidney** Protein Explorer

Cat # RKWB-81; (Rat: Sprague-Dawley, ~ 8 wks old, mixed gender)

Related Products:

1. Mouse monoclonal **beta-actin antibody**, cat # ACTB12-M; \$245/100 µg
2. **Western blot recycling kit** (strip antibodies in ~15 min. at room temp and re-use blots; sufficient reagents to strip 20-40 mini blots), Cat # 90100, \$195.
3. **Western blot kit** (contains all necessary blocking, wash, antibody dilute, ECL reagents and a specified (anti-rabbit, mouse etc antibody conjugates; sufficient for 15-30 blots), Cat # 80200,
4. High range **multi-colored mol. Wt markers** as shown on the blot, Cat # HMWM-11; (load 5-10 µl/lane).

ReadyBlot Kidney Protein Explorer

Adult Rat Kidney, Cat. No. RKWB-81

Study distribution of proteins in 6 anatomically and functionally defined regions of rat kidney with premade protein blots



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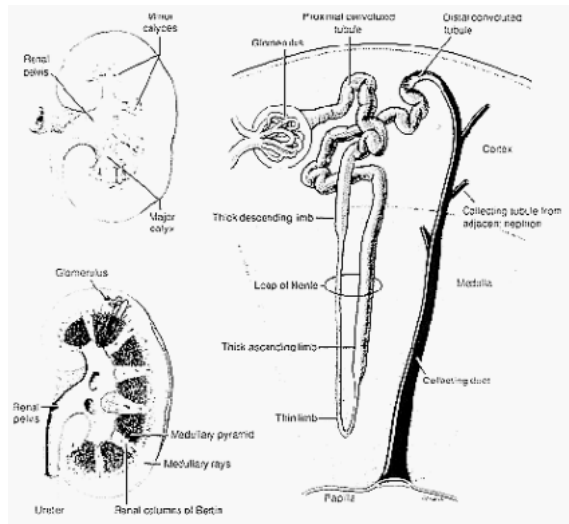
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The kidney is responsible for several homeostatic functions, including the regulation of extracellular fluid volume, K⁺ balance, acid-base homeostasis and regulation of the tonicity of the body fluid. To perform these homeostatic functions, the kidneys regulate NaCl reabsorption, K⁺ secretion, net acid excretion, and water transport. The presence, the distribution and the localization of highly specialized ion or water transport pathways along different segments of the nephron are fundamental to the response of kidney to changes in water or solutes intake.



A coronal section of the kidney shows the following structural regions (Fig. 1):

Cortex: as a whole, is cup-shaped, has inverted margins, and surrounds the renal medulla. In addition to the Glomerulus, Cortex contains the following nephron segments: Proximal tubule (with S1, S2 and 50% of S3 segments), 10 to 20% of Cortical Thick ascending limb (in the superficial cortex), distal tubule, and cortical collecting duct.

Outer Medulla: can be divided into 2 regions: Outer Stripe linked to the cortex, and Inner Stripe far from Cortex, and linked to the inner medulla. Inner Stripe of Outer medulla, which contain 100% of medullary thick ascending limb (mTAL)

tubules, Thin descending limb, and Outer Medullary collecting duct (OMCD). Inner Medulla or the **papilla** projects into the renal pelvis. The papilla contains both Inner medullary collecting duct (IMCD) and the thin descending and ascending limb of Henle's loop. Majority (70-80%) of water, electrolytes (Na⁺, Cl⁻, K⁺, HCO₃⁻, Pi, etc) and organic solutes (Glucose, Amino acid, etc) filtered by the glomerulus are reabsorbed by the proximal tubules cells (localized in the cortex).

BLM and BBM are prepared from kidney cortex by Percoll density gradient method.

Acquisition of animal or human kidney tissue is not only time-consuming and expensive, but also requires expertise and training in kidney anatomy, cell and molecular biology. ADI has carefully dissected and processed anatomically and functionally distinct areas of kidney for the study of proteins using Western blots. The kidney proteins have been electrophoresed, electro-blotted, and blocked. A lane of pre-stained mol. wt markers is included in each blot to assist you in identifying the size of the proteins.

Each Kidney ReadyBlot has the proteins from the following regions:

Lane 1: multi-colored Mol. Wt markers (see details below).

Lane 2: Whole Kidney

Lane 3: Outer Cortex

Lane 4: Inner Cortex

Lane 5: Medulla

Lane 6: Papilla

Lane 7: Basolateral Membranes (BLM)

Lane 8: Brush Border Membranes (BBM)

Lane 8: Brush Border Membranes (BBM)

Protein Load: ~20 µg protein; Further optimized for equal protein load (Fig 2) and with beta-actin (Fig. 3) immuno blot.

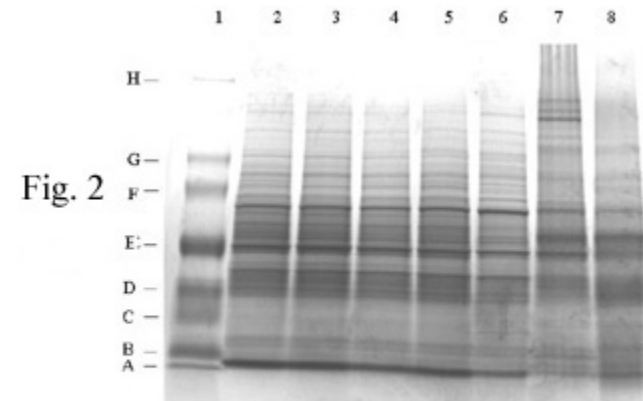


Fig. 3

The proteins representing the total extracts of different regions of the kidney were stained with comassie (Fig. 2) or probed with beta-actin antibody (Fig. 3).