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 quiet 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 is PBS+azide if blots are to be re-used (stripped) to study with another antibody.

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

An attempt has been made to equalize the protein load with beta-actin. However, antibody reactivity with beta-actin in various tissues may differ due to selective posttranslational modifications) or the fact that the antibody may not react with certain actin-isoforms (e.g., muscle). It is also important to realize that there is NO protein that remains the same in all physiological or pathological conditions. But beta-actin, tubulin, or glyceraldehydes-3-phosphate dehydrogenase has often been used as controls.

Ordering Information

Adult **Rat** ReadyBlot **Tissue** Protein Explorer
Cat # RTWB-91; $495.00 (Rat: Sprague-Dawley, ~ 8 wks old, mixed gender)

Please inquire about the availability of 10 additional tissue protein blots

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Adult **Mouse** ReadyBlot **Tissue** Protein Explorer
Cat # MTWB-71; $495.00 (Mouse: Swiss Webster, ~10 wks old, mixed gender)

Please inquire about the availability of 10 additional tissue protein blots

Related Products:

1. Mouse monoclonal **beta-actin antibody**, cat # ACTB12-M; $245/100 ug

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, $295 per kit.

4. High range **multi-colored mol. Wt markers** as shown on the blot, Cat # HMWM-11; $95.00/250 ul (load 5-8 ul/lane).

Instruction Manual No. RTWB-91

ReadyBlot Adult RAT Tissues Protein Explorer

Cat. No. RTWB-91

Study distribution of proteins in 10 major tissues of rat with premade protein blots

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Studies on distribution of a given protein in various tissues are carried out to find its probable function. In addition, the protein levels may vary according to the age or physiological or pathological conditions. There is also evidence of selective processing of mRNA (alternative splicing, posttranslational modifications (glycosylation, phosphorylation, etc) or interaction with other modifying partners. Therefore, mRNA and protein levels may not always correlate. It is important to study the actual levels of a given protein in various tissues. However, acquisition of animals, preparation of appropriate protein extracts, and subsequent processing of protein gels is not only time-consuming and expensive, but also requires expertise and training in tissue processing and preparation of protein samples. In order to simplify and expedite this process, ADI has carefully dissected and processed several major tissues from rat and mouse. Total tissue proteins have been, extracted, 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. These blots can be part of an initial feasibility study to see if there is an interesting aspect related to a protein distribution. A more detailed, controlled study can then be initiated to elaborate initial findings.

ReadyBlot protein explorer has the proteins from the following tissues:

Lane 1: multi-colored Mol. Wt markers (see details below).
Lane 2: Brain **Lane 3**: Heart **Lane 4**: Kidney
Lane 5: Liver **Lane 6**: Lung **Lane 7**: Skeletal muscle
Lane 8: Pancreas **Lane 9**: Spleen **Lane 10**: Testis
Lane 11: Thymus

Tissue Processing: Freshly dissected and washed tissues were homogenized in an isotonic extraction buffer (Tris buffer pH 7.5, containing EDTA, sucrose and proprietary additives including several protease inhibitors), centrifuged to remove debris and nuclei, and clear supernatants (extracts) collected. The tissue extracts should contain most cytoplasmic and membrane proteins. Protein was measured and equalized with respect to total proteins (Fig. 1) and beta-actin (Fig. 2) immunostaining.

SDS-Gel Electrophoresis and blotting: Tissue protein extracts were mixed with 2X standard Laemmli **reducing** buffer, heated for 5 min at 90oC. Approx **30 ug total proteins** were run on **4-20%-reducing SDS-mini gels** at 200 V for approx. 45 min. Pre-stained **high range mol. Wt markers** (ADI Cat # HMWM-11) were loaded on each gel. The position of markers from bottom to top is:

Marker #	Protein	Marker Color	Mol Wt kDa
A	Aprotinin	Blue	6.5
B	Lactalbumin	Purple	14.2
C	Soybean trypsin inhibitor	Green	20
D	Carbonic anhydrase	Orange	29
E	Ovalbumin	Yellow	45
F	Bovine serum albumin	Pink	66
G	Beta-galactosidase	Turquoise	116
H	Muscle myosin	Blue	205

The proteins were transferred to PVDF membrane using mini-transblot cells. Homogeneity of protein transfer in all 11 lanes was verified using water soluble Stain-ALL (ADI Cat # SALL-500) for 5 min. Protein lanes were identified and marked 1-11. 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.

Fig. 1.

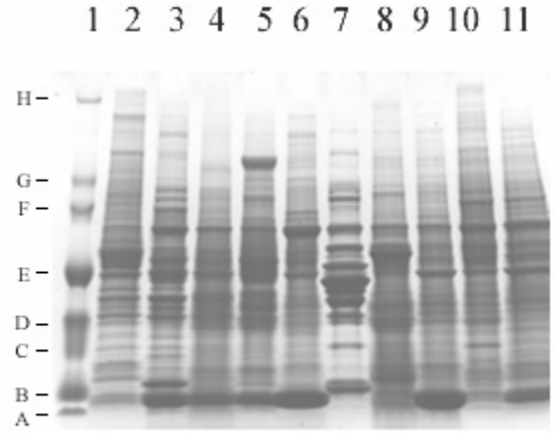

Fig. 2.

Total protein profile of **adult rat tissue** stained with comassie blue (Fig. 1). Adult **rat tissue proteins** probed with beta-actin antibody (Fig. 2).