

Form, Storage, and Recommended Usage:

Store unused blots at 4°C in a sealed bag.
Blots are blocked with milk-based buffer (#80062). ReadyBlot should be handled with care. These blots should be used within 6 months.

It is recommended to wet the blot first in 100% methanol until the blot is wet followed by washing in PBS to remove methanol and the dye. The blot is ready to be incubated with appropriate antibodies. The standard Western protocol can be used. After the blot usage, it is recommended to keep the blot in 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 cells. 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 cells 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 (Human, Mouse, Rat Protein Blots)

HCWB-15 **Human Tumor Cells** (9) ReadyBlot tissue Protein Explorer
HTWB-10 **Adult Human Tissues** (11) ReadyBlot tissue Protein Explorer

MBWB-11 ReadyBlot **Brain** Protein Explorer (**Adult Mice**)
MBWB-12 ReadyBlot **Brain** Protein Explorer (**Newborn Mice**)
MBWB-13 ReadyBlot **Brain** Protein Explorer (~18-24 months **Old Mice**)

RBWB-11 ReadyBlot **Brain Protein Explorer** (Adult Rat)
RBWB-12 ReadyBlot **Brain** Protein Explorer (**Newborn Rat**)
RBWB-13 ReadyBlot **Brain** Protein Explorer (~18-24 months **Old Rat**)

MDWB-41 **Adult Mouse Digestive Tract** ReadyBlot Protein Explorer
MDWB-42 **Newborn Mouse Digestive Tract** ReadyBlot Protein Explorer
RDWB-51 **Adult Rat Digestive Tract** ReadyBlot Protein Explorer
RDWB-52 **Newborn Rat Digestive Tract** ReadyBlot Protein Explorer

MTWB-71 **Adult Mouse Tissues** (10) ReadyBlot tissue Protein Explorer
MTWB-72 **Newborn Mouse Tissues** (10) ReadyBlot tissue Protein Explorer
RTWB-91 **Adult Rat Tissues** (10) ReadyBlot tissue Protein Explorer
RTWB-92 **Newborn Rat Tissues** (10) ReadyBlot tissue Protein Explorer

RKWB-81 **Adult Rat** ReadyBlot **Kidney** Protein Explorer
MKWB-61 **Adult Mouse** ReadyBlot **Kidney** Protein Explorer

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

Instruction Manual No. HTWB-10

ReadyBlot Adult Human Tissues Protein Explorer

Cat. No. HTWB-10

Study distribution of proteins in 11 major tissues of human 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 human. 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 Human Tissue protein explorer has proteins from the following tissues:

- Lane 1: Mol. Wt markers (see details below).
- Lane 2: Brain Lane 3: Heart Lane 4: small intestine
- Lane 5: Kidney Lane 6: Liver Lane 7: Lung
- Lane 8: Sk. Muscle Lane 9: Stomach Lane 10: Spleen
- Lane 11: Ovary Lane 12: testis

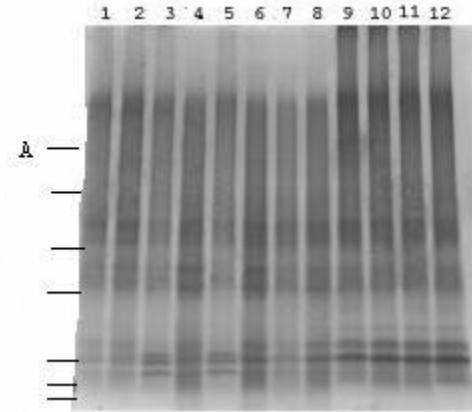
Tissue Processing: Tissues samples (post-mortem) were obtained after informed consent. Tissues were stored frozen at -80oC until processed. Tissues were homogenized in an isotonic extraction buffer (Tris buffer pH 7.5, containing EDTA, sucrose and proprietary additives including several protease inhibitors), 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 **10 ug total proteins** were run on **4-20%-reducing SDS-mini gels** at 200 V for approx. 45 min. **High range mol. Wt markers** were loaded on each gel. The position of markers from top to bottom is:

Markers (kda): **A** (top) 180; **B**, 116; **C**, 97; **D**, 66; **E**, 48; **F**, 29; **G**, 18; and **H**, 14.

The proteins were transferred to **PVDF** membranes using mini-transblot cells. Homogeneity of protein transfer in the sample was verified using water soluble amido-black dye. Protein lanes were identified and marked 1 & 12. Membranes were washed in PBS to remove the dye. **Multi-colored Mol. Wt. Standards (Lane 1) have also been marked A-H on the blot.**

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



Total protein profile of **adult human tissues. PVDF membranes stained with amido black..**

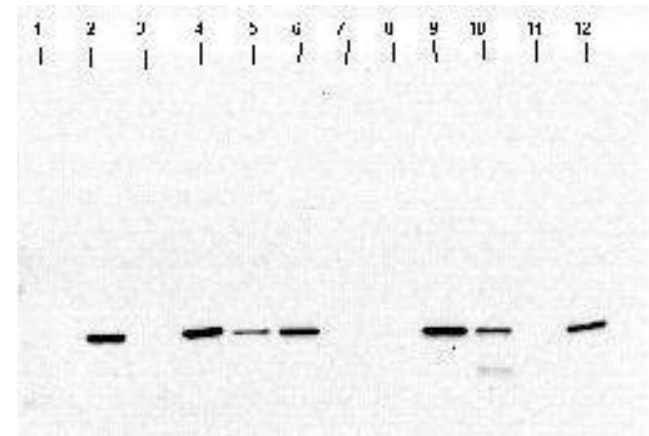


Fig. 2. Beta actin (~45 kda) profile of human tissue protein blot. Some tissues do not react with the non-muscle type beta-actin antibody used in this study.