SDS-Gel Electrophoresis and blotting: Digestive tract protein extracts were mixed with 2X standard Laemmli **reducing** buffer, heated for 5 min at 90°C**. The proteins** were run on **4-20%-reducing SDS-mini gels** at 200 V for approx. 50 min. Multi-colored **high range mol. Wt markers** (ADI Cat # HMWM-11) were loaded in on each gel: (Aprotinin, Blue, 6.5K); (Lactalbumin, purple, 14.2K); (Soybean trypsin inhibitor, green, 20.1); (Carbonic anhydrase, orange, 29K); (Ovalbumin, yellow, 45K); (bovine serum albumin, pink, 66K); (beta-glactosidase, turquoise, 116K), and (muscle myosin, blue, 205K). The proteins were transferred to PVDF membrane using mini-transblot cells. Homogeneity of protein transfer in all 14 lanes was verified using water soluble Stain-ALL (ADI Cat # SALL-500) for 5 min. Protein lanes were identified and marked 1-14. 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 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.

This blot will be most useful for proteins that are relatively abundant in a particular region of digestive tract. Very low abundant proteins that require the use of enriched cell membranes, cytosolic or nuclear fractions may be poorly represented in this blot.

Ordering Information

Newborn **Mouse** ReadyBlot **Digestive Tract** Protein Explorer
Cat # *MDWB-42***; $495.00 (**Mouse: Swiss Webster, ~8-10 days old, mixed gender)

Please inquire about the availability of 15 additional tissue protein blots.

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Adult **Rat** ReadyBlot **Digestive Tract** Protein Explorer
Cat # *RDWB-51*; **$495.00** (Rat: Sprague-Dawley, ~8 wks old, mixed gender)
Adult **Mouse** ReadyBlot **Digestive Tract** Protein Explorer
Cat # *MDWB-41***; $495.00 (**Mouse: Swiss Webster, ~10 wks old, mixed gender)

Related Products:

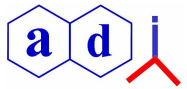
1. Mouse monoclonal **beta-actin antibody**, cat # ACTB12-M; $245/100 µg
2. **Western blot recycling kit** (strip antibodies in ~15 min. at room temp and re-use blots; sufficient reagents to strip 20-40 mini blots), Cat # 90100, $195.
3. **Western blot kit** (contains all necessary blocking, wash, antibody dilute, ECL reagents and a specified (anti-rabbit, mouse etc antibody conjugates; sufficient for 15-30 blots), Cat # 80200, $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 µl/lane).

Instruction Manual No. MDWB-42

ReadyBlot Digestive Tract Protein Explorer

Newborn Mouse Digestive Tract; Cat. No. ***MDWB-42***

Study distribution of proteins in 13 anatomically and functionally defined regions of digestive tract with premade protein blots



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The digestive system consists of digestive tract and its associated glands. The gastrointestinal tract can be divided into the following major regions: **Esophagus; Stomach** (Cardia, Fundus, Body and Pylorus); **Small Intestine** (Duodenum, Jejunum and Ileum); **Large Intestine** (Cecum, Colon, Rectum, and Appendix). The entire tract is primarily composed of a lumen, which is surrounded by a wall made up of 4 principal layers: **mucosa** (mucous membrane), **submucosa**, **muscularis externa** and **serosa**. Each layer contains glands, blood and lymph vessels and lymphoid tissues The **mucosa** is composed of epithelial lining, lamina propria (a loose connective tissue) and two layers of smooth muscle cells (*muscularis mucosae*). The **submucosa** is composed of loose connective tissue with many submucosal nerve plexus. The **muscularis externa** contains 2 layers of smooth muscle cells with *myenteric nerve plexus,* sandwiched in-between. The **serosa** is composed of one inner layer of a loose connective tissue rich in adipose tissue and an outer squamous epithelial layer of covering (*mesothelium*). The **Esophagus**, is a muscular tube through which, food-stuff is transported from mouth to stomach. It is covered by nonkeratinized, stratified squamous epithelium. It contains mucus secreting *esophageal* and *esophageal cardiac glands* in the submucosa and lamina propria layers, respectively. The **Cardia**, is a narrow circular band at the transition between esophagus and stomach. Its lamina propria contains *tubular cardiac glands*. Its parietal cells secrete HCl and other secretory glands produce mucus and lysozyme. The **Fundus** and the **Body**, contain branched tubular *gastric glands* in lamina propria. These glands consist of undifferentiated, mucous neck, parietal (Oxyntic), chief (Zymogenic) and enteroendocrine cells. The secretions from these cells include HCl, KCl and traces of other electrolytes, pepsin, lipase and a gastric intrinsic factor (a glycoprotein which binds avidly to vitamin B12). In fundus of the stomach, 5-hydroxytryptamine (serotonin) is one of the principal secretory products. The **Pylorus**, contain tubular *pyloric glands* secreting mucus, gastrin and lysozyme. The enteroendocrine cells of pylorus secrete *somatostatin,* which inhibits the release of other hormones, including gastrin. The **Small Intestine** begins at **Duodenum**. The duodenum commences at the pylorus and unites with at **Jejunum**. Most of the lipid absorption occurs in the duodenum and upper jejunum. The lining of small intestine has a series of permanent folds (*plicae circulares*) consisting of mucosa and submucosa. The *plicae circulares* are charesteristics of **jejunum** but not of **duodenum** or **ileum**. The small intestine also contains *intestinal glands* (crypts or glands of Lieberkuhn) and *Intestinal villi* which are outgrowths of the mucosa. The intestinal glands consist of several types of cells including undifferentiated, goblet, Paneth, enteroendocrine, membranous epithelial (M cells) and absorptive (microvilli) cells. M-cells endocytize and transport antigens to lymphoid cells to initiate the immune response to foreign antigens. The intestinal submucosa contains numerous tubular *Duodenal glands* (Brunners glands), comprised of mucous type cells. These glands secrete a polypeptide, urogastrone, which inhibits gastric acid secretion and stimulates cell proliferation. The lamina propria submucosa also contain lymphoid nodules (Peyer's patches) which are found, mostly, in the ileum. The **Large Intestine** extends from the end of the ileum to the anus. It differs from small intestine in its greater caliber and the presence of appendages (**Appendices epiploicae**). The **Cecum**, commencement of the large intestine, is a large blind pouch. The open end of cecum communicates directly with the **Proximal Colon**, which is smaller in caliber than cecum. The **Distal Colon** extends from the proximal colon and is a highly movable part of the colon . It differs from small intestine in its greater caliber and the presence of appendages (Appendices epiploicae). It is almost completely invested by the peritoneum. and ends into rectum. The **Rectum** is continuous above with colon and ends in the anal canal.

The large intestine is responsible for passive absorption of water and formation of fecal mass. It consists of a mucosal membrane with no folds except in the **Rectal** portion. The cells of epithelial lining have short microvilli and Liberkuhn glands consist of large number of globlet cells, absorptive cells and a small number of enteroendocrice cells. The muscularis externa is composed of longitudinal strands which congregate into three thick bands called *Teniae coli*.

Acquisition of animal or human digestive tract tissue is not only time-consuming and expensive, but also requires expertise and training in anatomy, cell and molecular biology. ADI has carefully dissected and processed 13 anatomically and functionally distinct areas of digestive tract for the study of proteins using Western blots. The proteins have been electrophoresed, electro-blotted, and blocked.

Each Digestive Tract ReadyBlot has the proteins from the following regions:

Lane 1: Multi-colored Mol. Wt markers (see details below).		**Lane 2**: Esophagus
Lane 3: Cardia	**Lane 4**: Fundus	**Lane 5**: Body
Lane 6: Pylorus	**Lane 7**: Duodenum	**Lane 8**: Jejunum
Lane 9: Ileum	**Lane 10**: Cecum	**Lane 11**: Proximal Colon
Lane 12: Distal Colon	**Lane13**: Rectum	**Lane14**: Liver

Protein Load: ~40 µg protein; Further optimized for equal protein load (Fig 1) and with beta-actin (Fig. 2) immuno blot.

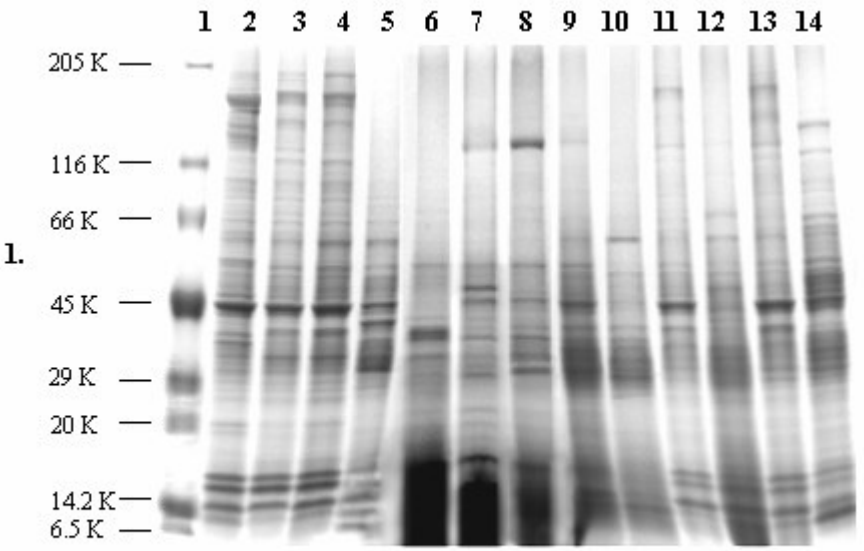

Fig. 1.

Fig. 2.

The proteins representing the extracts of different regions of the digestive tract were stained with comassie (Fig. 1) or probed with beta-actin antibody (Fig. 2).