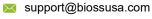


+1.781.569.5821 [INT'L]



# Rat TFF3 Ready-To-Use IHC Kit

Cat. No.: IHC0103R

🚱 www.biossusa.com

Sample Type: FFPE tissue Size: 50T (including a control slide)

Storage and Stability: Please store components at the temperatures indicated on the individual tube

labels. The kit is stable for 6 months from the date of receipt.

#### **General Information**

Number	Component	Size	Concentration	Storage
1	PBS Buffer (powder)	2 L×2	20x	RT
2	Antigen Retrieval Buffer	20 ml	100x	2-8℃
3	Endogenous Peroxidase Blocking Buffer	3 ml	RTU	2-8℃
4	Blocking Buffer	3 ml	RTU	2-8℃
5	Primary Antibody (Rat TFF3 Rabbit pAb)	6 ml	RTU	2-8℃
6	Secondary Antibody (HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8℃
7	Chromogen Component A	0.3 ml	RTU	-20℃
8	Chromogen Component B	0.3 ml	RTU	<b>-20</b> ℃
9	Counter Staining Reagent	5 ml	RTU	RT
10	Mounting Media	5 ml	RTU	RT
11	Control slide (Rat small intestine)	1 slide	RTU	RT
12	Datasheet	1 сору		

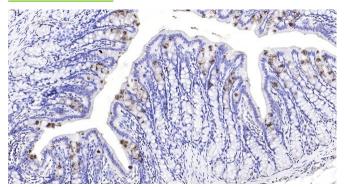
### **Background**

Trefoil peptides are protease resistant molecules secreted throughout the gut that play a role in mucosal healing. Trefoil peptides contain three intrachain disulfide bonds, forming the trefoil motif, or P-domain and clustered in human chromosome 21q22.3. Trefoil Factor 3 (TFF3) is one of three trefoil peptides secreted by epithelial cells that line mucus membranes in the large and small intestines and lower respiratory tract. TFF3 is mainly active as a disulfide-linked homodimer and may have a role in promoting cell migration in healing processes. It is involved in the maintenance and repair of the intestinal mucosa.

#### **Synonyms**

Trefoil Factor 3; Trefoil factor3; hP1.B; Intestinal trefoil factor; ITF; mITF; OTTMUSP00000021729; Polypeptide P1.B; TFF3; TFI; TREFOIL; Trefoil factor (intestinal).

#### **Validation Data**



Immunohistochemical analysis of paraffin embedded rat colon tissue slide using IHC0103R (Rat TFF3 IHC Kit).

# **Immunohistochemistry Protocol**

#### 1. Deparaffinization And Rehydration

Immerse slides in fresh xylene for 15 minutes and then repeat two more times using separate containers. Immerse slides sequentially in 100%, 95%, 90%, 80%, and 70% ethanol solutions for 5 minutes each. Rinse slides 3 times with distilled water for 5 minutes each.

#### 2. Antigen Retrieval

Add  $100 \times$  **Antigen Retrieval Buffer** into distilled water to prepare a  $1 \times$  solution. Boil slides in  $1 \times$  solution at  $95^{\circ}$ C- $100^{\circ}$ C for 15 minutes. Move the slides to  $1 \times$  solution at room temperature (RT) and allow them to stand for 20 minutes. Rinse 3 times with **PBS Buffer** (dissolve the powder in 2L distilled water) for 5 minutes each.

#### 3. Block Endogenous Peroxidase

Drain the liquid off the slides and then use a hydrophobic IHC pen to draw circles on the slides around tissue sections. Add 2-4 drops of **Endogenous Peroxidase Blocking Buffer** directly on slides, covering the whole tissue and block slides for 15 minutes at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

#### 4. Serum Blocking

Block with 2-4 drops of **Blocking Buffer** for 20 minutes at RT.

# 5. Primary Antibody Incubation

Drain blocking buffer from slides. Incubate slides with 2-4 drops of **Rat TFF3 Rabbit pAb** overnight at 4°C or 1-2 hours at RT. Rinse 3 times with **PBS Buffer** for 5 minutes each.

#### 6. Secondary Antibody Incubation

Incubate slides with 2-4 drops of **HRP-Goat anti-Rabbit IgG pAb** for 1-2 hours at RT. Rinse slides 3 times with **PBS Buffer** for 5 minutes each.

#### 7. Signal Development

Remove residual liquid around the tissue section. Add 50ul fresh **DAB Buffer** (**Chromogen Component A**: **Chromogen Component B**: **PBS Buffer=1:1:18**) to cover the tissue. Monitor the reaction under the microscope until a brown color is visible (approximate 3-5 minutes at RT). Stop reaction immediately by rinsing with distilled water. Rinse slides 3 times with distilled water for 5 minutes each.

#### 8. Counterstain

Counterstain with an appropriate amount of **Counter Staining Reagent** for 3-5 minutes at RT. Rinse slides with distilled water for 5 minutes. Use 2-4 drops of **Differentiation reagent** to cover the tissue for 30 seconds. Rinse slides twice with distilled water for 5 minutes each.

#### 9. Dehydration Sheet

Immerse slides sequentially in 70%, 80%, 90%, 95%, and 100% ethanol for 5 minutes each at RT. Immerse slides in 2 changes of fresh xylene, 15 minutes each. Drop some **Mounting Media** on the tissue. Mount coverslips.

#### **Notes**

- 1. The positive control slide provided in the kit allows you to be sure that the experimental set-up is working properly.
- 2. Do not allow slides to dry at any time during this procedure.
- 3. Please don't replace the matching reagents in this product with other manufacturers' products.
- 4. As DAB is a carcinogen, please take necessary precautions.
- 5. PBS (reagent 1) can be stored for one week at 4℃ after preparation; The antigen retrieval buffer (1×reagent
- 2) and the chromogenic agent (the mixture of reagents 7 and 8) should be prepared right before each assay.

Please cite this product as "IHC0103R, Bioss Antibodies". Citation example: "Rat tissue sections using Rat TFF3 IHC Kit (IHC0103R, Bioss Antibodies) were stained for TFF3 according to the manufacturer's instructions.



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# Rat TFF3 Ready-To-Use IHC Kit 大鼠三叶肽因子 3 即用型免疫组化试剂盒

产品货号: IHC0103R

样本类型: FFPE 组织切片 产品规格: 50T (包含一个对照切片)

保存条件:见下表。有效期6个月。

#### 产品组分及规格

编号	组分	规格	浓度	储存
1	PBS 缓冲液(干粉)	2 L×2	20x	室温
2	抗原修复缓冲液	20 ml	100x	2-8℃
3	内源性过氧化物酶阻断剂	3 ml	RTU	2-8℃
4	封闭工作液	3 ml	RTU	2-8℃
5	一抗(Rat TFF3 Rabbit pAb)	6 ml	RTU	2-8℃
6	二抗(HRP-Goat anti-Rabbit IgG pAb)	6 ml	RTU	2-8℃
7	DAB kit(20×)显色液	0.3 ml	RTU	-20℃
8	DAB kit(20×)稀释液	0.3 ml	RTU	-20℃
9	复染试剂	5 ml	RTU	室温
10	封片剂	5 ml	RTU	室温
11	对照切片 (大鼠小肠)	1 张	RTU	室温
12	说明书	1份		

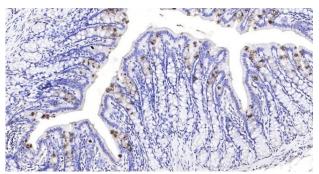
#### 背景

Trefoil peptides are protease resistant molecules secreted throughout the gut that play a role in mucosal healing. Trefoil peptides contain three intrachain disulfide bonds, forming the trefoil motif, or P-domain and clustered in human chromosome 21q22.3. Trefoil Factor 3 (TFF3) is one of three trefoil peptides secreted by epithelial cells that line mucus membranes in the large and small intestines and lower respiratory tract. TFF3 is mainly active as a disulfide-linked homodimer and may have a role in promoting cell migration in healing processes. It is involved in the maintenance and repair of the intestinal mucosa.

# 别名

Trefoil Factor 3; Trefoil factor3; hP1.B; Intestinal trefoil factor; ITF; mITF; OTTMUSP00000021729; Polypeptide P1.B; TFF3; TFI; TREFOIL; Trefoil factor (intestinal).

#### 验证数据



使用 IHC0103R(大鼠三叶肽因子 3 IHC 试剂盒)对石蜡包埋的大鼠结肠组织切片进行免疫组化分析。

# 石蜡包埋组织的免疫组织化学方案

#### 1. 脱蜡水化

石蜡切片置于新鲜二甲苯中浸泡脱蜡 3 次,每次 15 min;依次置于不同浓度(100%、95%、90%、80%、70%) 乙醇浸泡各 5 min,再置于蒸馏水洗涤 5 min,重复 3 次。

#### 2. 抗原修复

沸水浴修复:将 100×抗原**修复缓冲液(试剂 2)**用蒸馏水稀释成 1×抗原修复缓冲液,放入修复盒中并提前加热至 95-100  $\mathbb{C}$  (注意盖好以防液体蒸发),然后将切片放入修复盒中,在沸水浴环境中保持外沸状态 15 min,室温自然 冷却,用 **PBS 缓冲液(试剂 1**,将干粉溶解在 2L 蒸馏水中)清洗 5 min,重复 3 次。

# 3. 阻断内源性过氧化物酶

用吸水纸吸去玻片上多余的液体,用免疫组化笔在组织周围画圈,加入 2-4 滴**内源性过氧化物酶阻断剂(试剂 3)**, 室温下置于湿盒中孵育 15 min, 用 PBS 洗涤 5 min, 重复 3 次。

#### 4. 血清封闭

用吸水纸吸去玻片上多余的液体,加入 2-4 滴**封闭工作液(试剂 4)**,置于湿盒内 37℃封闭 20 min,以减少非特异性染色。

# 5. 一抗孵育

用吸水纸吸去玻片上多余的液体,加入 2-4 滴**大鼠 TFF3 兔多抗工作液(试剂 5)**,置于湿盒中,4 $^{\circ}$ 孵育过夜或 37 $^{\circ}$ m f 1-2 h。

#### 6. 复温

4℃孵育过夜后,室温下复温 15 min(若在室温下孵育一抗,则直接进入下一步清洗),用 PBS 洗涤 5 min,重复 3 次。

#### 7. 二抗孵育

用吸水纸吸玻片上多余的液体,加入 2-4 滴 **HRP 标记羊抗兔 lgG 工作液 (试剂 6)**,置于湿盒中, $37^{\circ}$ 则解育 1-2 h;用 PBS 洗涤 5 min,重复 3 次。

#### 8. 显色

用吸水纸吸去玻片上多余的液体,在每张切片上滴加约 50  $\mu$ L 新配制的 **DAB 工作液(试剂 7:试剂 8:PBS=1:1:18)**,作用 3-5 min。显微镜下观察结果,达到合适的显色强度后,用蒸馏水冲洗切片以终止反应,用蒸馏水冲洗 5 min,重复 3 次。

#### 9. 复染

滴加适量**复染试剂(试剂 9)**复染 3-5 min,蒸馏水冲洗 5 min,滴加盐酸酒精分化约 30 s,蒸馏水洗涤 5 min,重复 2 次。

#### 10. 脱水封片

将玻片依次置于不同浓度(70%、80%、90%、95%、100%)乙醇,各 5 min;然后置于新鲜二甲苯中浸泡脱蜡 3 次,每次 15 min。用吸水纸吸去多余的二甲苯,滴加适量**封片剂(试剂 10**)在组织上,将盖玻片盖在组织上,避免产生气泡。

#### 注意事项

- 1. 建议检测时进行阴性及阳性对照,以提高实验的可靠性。
- 2. 本品中的配套试剂,请不要用其他生产商产品替换使用。
- 3. DAB 为致癌物质,请采取必要的防范措施。
- 4. PBS 洗涤液(试剂 1)配制后在 4 ℃可保存一周;抗原修复液(试剂 2)及显色剂(试剂 7 和 8)的工作液需每次实验时现用现配。
- \*5. 发表论文时引用本产品的写作建议 "IHC0103R, Bioss Antibodies"。引用示例: "Rat tissue sections using Rat TFF3 IHC Kit (IHC0103R Bioss Antibodies) were stained for TFF3 according to the manufacturer's instructions."