清准医学
TP MedTech

产品说明书 USER GUIDE

VIVIT免疫荧光染色含脂肪组织透明化试剂盒

产品信息

VIVIT组织透明化试剂盒系列产品基于创新的离子液体透明化技术，开辟了组织折射率匹配的第三种机制，实现了“高透明”与“微形变”兼得。同时，其具有增强荧光和低温下不形成冰晶的独特优势(Cell, 2025)，为离体厚组织三维成像提供了创新型解决方案。试剂盒含脱脂试剂、过离子化试剂及离子液体匹配试剂等核心组分，可将组织转变为离子玻璃态，实现高透明度组织透明化，并使该组织能够在低温下(-80℃) 长期储存、避免荧光信号衰减。VIVIT处理后的组织兼容各类三维荧光成像方式，操作便捷，结果稳定，能够为科研组织分析提供可靠工具。

对于免疫荧光染色后的内脏等富含脂肪组织，推荐使用VIVIT免疫荧光染色含脂肪组织透明化试剂盒。

试剂名称与储存条件

试剂	规格	说明	储存条件
抗体交联剂（10X）	1 mL*3	用于对抗体染色后的组织进行抗体交联固定	-80℃或-20℃
	1 mL*1		
脱脂试剂A2 (DIDC-C)	30 mL	用于组织脱脂	常温储存；最佳储存温度：-20℃
	10 mL		
脱脂试剂B	30 mL	用于富含脂肪组织的梯度脱脂	常温储存
	10 mL		
过离子化试剂B (SDS)	30 mL	用于富含脂肪组织的过离子化处理	常温储存；最佳储存温度：-20℃
	10 mL		
离子液体匹配试剂 (RIL-C)	30 mL	用于漂洗的离子液体溶液	最佳储存温度：-80℃；请勿置于4℃环境
	10 mL		
离子液体匹配试剂 (IL5-C)	30 mL	离子液体匹配试剂 折射率：1.500-1.508	
	10 mL		
离子液体匹配试剂 (IL2.5-C)	30 mL	离子液体匹配试剂 折射率：1.538-1.545	
	10 mL		

未提供的必要材料

0.01 M PBS（磷酸盐缓冲液）

样本处理指南

组织透明化前需进行被动浸泡的组织固定或经心灌流的组织固定；推荐使用4%PFA经心灌流进行组织固定固定后请用0.01 M PBS充分漂洗组织以去除残留的PFA。本产品用于透明化免疫荧光染色后的厚组织切片，免疫荧光染色可通过常规免疫荧光染色流程或厚组织免疫荧光染色流程（如whole-mount、SWITCH、eFLASH等）进行。透明化处理包括脱脂、过离子化、折射率匹配等环节。

处理过程中如遇问题，可参考【注意事项】。

具体步骤如下(适用于厚度 1mm的厚组织，更薄可酌情减少处理时间，更厚可酌情增加处理时间)：

1. 抗体交联：将完成免疫荧光染色的组织转移到用0.01 M PBS溶液稀释10倍的抗体交联液中，4℃孵育8-12 h。
2. 组织脱脂：将组织转移至脱脂试剂A2中，在摇床上37℃孵育2-4h。随后将组织转移20%脱脂试剂A2-80%脱脂试剂B的混合液中，常温孵育2-4h。随后将组织转移脱脂试剂B中，常温孵育2-4h。最后将组织转移至脱脂试剂A2中，37℃孵育2-4h。可根据处理效果适当延长处理时间。
3. 过离子化处理：弃去容器中的脱脂试剂A2，加入过离子化试剂B，在摇床上常温孵育4 h。
4. 漂洗：弃去容器中的试剂，更换成提前平衡至室温的离子液体匹配试剂RIL-C，加入后立刻颠倒混匀。在摇床上冰浴漂洗1h，推荐处理过程中额外上下颠倒、手动混匀若干次。
5. 折射率匹配：弃去容器中的试剂，更换成离子液体匹配试剂IL5-C，加入后立刻颠倒混匀，在摇床上室温孵育2-4h，推荐处理过程中额外上下颠倒、手动混匀若干次。随后弃去容器中的试剂，更换成离子液体匹配试剂IL2.5-C，加入后立刻颠倒混匀，在摇床上室温孵育4-6h，推荐处理过程中额外上下颠倒、手动混匀若干次，直至组织完全透明。

注意事项

- 本试剂盒仅适用于离体组织的透明化处理，不可用于在体组织。
- 本试剂盒保质期一年，生产日期见产品标签。
- 所有试剂如果在低温存储应平衡到室温再开盖使用，若1周内使用可储存于室温。
- 抗体交联剂到货后建议分装储存，该试剂吸潮后易失效；用PBS稀释后请立即使用，稀释后的液体常温存放2 h后严重失效。
- IL2.5-C、IL5-C置于4℃或-20℃环境可能析出晶体；-80℃存储可以避免上述试剂析出晶体。
- 若出现IL5-C和IL2.5-C试剂析出的情况，可将本试剂严格密封，置于50℃水浴/金属浴环境中，直至晶体溶解，大约需要30分钟，完全溶解后可正常使用。
- 可根据每一步处理效果适当延长相应步骤的处理时间。通常地，组织更厚应额外增加各步骤处理时间，长时间固定样本也应该额外增加各步骤处理时间。
- 为保证透明化质量，组织浸入试剂后需使用摇床或间断手动混匀。
- 每片1 cm^2 1 mm厚组织推荐试剂用量约为3 mL，用量过少可能会影响透明化效果。
- 经过 VIVIT 处理的组织可以在 -80℃下长期储存。
- 透明化处理暂停点可设置于除离子液体匹配试剂RIL-C漂洗以外的任意步骤，除离子液体匹配试剂RIL-C漂洗以外延长其余步骤处理时间几乎不影响透明化结果，建议脱脂、使用离子液体匹配试剂IL2.5-C折射率匹配环节过夜处理。
- DIDC-C很容易清除掉油性笔迹。使用时请盖紧盖子，防止漏液。
- 本产品仅限于专业人员的科学研究用，不得用于临床诊断或治疗。
- 为了您的安全和健康，使用试剂时请佩戴一次性手套及口罩操作。
- 所有试剂请远离火源及热源，于避光、阴凉处储存，避免阳光直射。
- 所有组织处理的废液均应单独收集于专用容器中，不得与强氧化剂混合，请勿将废液直接倒于下水道中。
- 针对初次使用用户，我们推荐按照说明书标注的用量和时间处理样本，并按需购买选配试剂。特殊需求用户请在官网联系我们寻求技术支持。

VIVIT IF Adipose-Rich Tissue Clearing Kit (Ionic Liquid-Based)

Product information

The VIVIT Tissue Clearing Kit series, leveraging innovative ionic liquid-based clearing technology, establishes a third mechanism for tissue refractive index matching, achieving both "high transparency" and "minimal deformation". It uniquely offers enhanced fluorescence and eliminates ice crystal formation at low temperatures (Cell, 2025), providing an innovative solution for 3D imaging of thick ex vivo tissues. The kit contains core components including delipidation reagent, overionization reagents, and ionic liquids matching reagent. These components transform tissues into an ionic glass-like state, achieving optical transparency, enabling highly transparent tissue clearing. Critically, tissues processed this way can be stored long-term at low temperatures (-80℃) without fluorescent signal decay. VIVIT-processed tissues are compatible with various 3D fluorescence imaging methods. Featuring user-friendly operation and stable outcomes, it provides a reliable tool for tissue analysis in scientific research.

For immunofluorescence-stained adipose-rich tissues, such as viscera, the VIVIT IF Adipose-rich Tissue Clearing Kit is recommended.

Reagents and Storage Condition

Reagent	Size	Notes	Storage
Antibody cross-linking reagent	1mL*3	For cross-linking and fixation of antibodies to tissues after	-80°C or -20°C
	1mL*1		
Delipidation reagent A2 (DIDC-C)	30 mL	For delipidation of tissue	Store at room temperature.
	10 mL		
Delipidation reagent B	30 mL	For gradient delipidation of adipose-rich tissue	Store at room temperature.
	10 mL		
Overionization reagents B (SDS)	30 mL	For overionization of adipose-rich tissue	Store at room temperature.
	10 mL		
Ionic liquids matching reagent (RIL-C)	30 mL	Ionic liquid solution for rinsing purposes	Optimal temperature: -80°C Do not store at 4°C.
	10 mL		
Ionic liquids matching reagent (IL5-C)	30 mL	Ionic liquids matching reagent	
	10 mL		
Ionic liquids matching reagent (IL2.5-C)	30 mL	Ionic liquids matching reagent	
	10 mL		

Required material not supplied

0.01 M PBS

Tissue Processing Guidelines

Prior to tissue clearing, tissue fixation should be performed either by passive immersion or by intracardiac perfusion. Perfusion fixation with 4% PFA is recommended. After fixation, thoroughly rinse the tissue with 0.01 M PBS to remove residual PFA. The kit is designed for ex vivo tissue clearing following immunofluorescence staining. The immunofluorescence staining can be performed using either conventional immunofluorescence staining protocols or thick tissue immunofluorescence staining methods (such as whole-mount, SWITCH, eFLASH, etc.).The tissue clearing process involves steps such as delipidation, overionization, and Ionic liquids matching. If issues arise during processing, refer to the [Precautions] section.

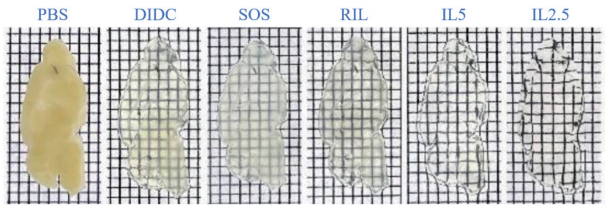
Specific steps (suitable for thick tissues of 1mm thickness; reduce processing time for thinner tissues and increase for thicker tissues as appropriate):

1. Antibody Cross-linking: Transfer the tissue that has completed immunofluorescence staining into the antibody cross-linking reagent (diluted 10-fold with 0.01 M PBS solution). Incubate at 4°C for 8-12 hours.
2. Tissue Delipidation: Transfer the tissue to delipidation solution A2 and incubate on a shaker at 37°C for 2-4 hours. Then transfer the tissue to a mixture of 20% delipidation solution A1 and 80% delipidation solution B, and incubate at room temperature for 2-4 hours. Finally, transfer the tissue to delipidation solution B and incubate at room temperature for 2-4 hours. The processing time may be appropriately extended based on the observed effect.
3. Tissue Overionization: Discard the delipidation reagent A2 in the container. Add overionization reagent B. Incubate on a shaker at room temperature for 4 hours.
4. Rinsing: Discard the reagent in the container and replace it with ionic liquid rinsing reagent RIL-C pre-equilibrated to room temperature. Invert to mix immediately after adding. Perform an ice-bath rinse on a shaker for 1 hour; additionally, inverting and manually mixing several times during this step is recommended.
5. Refractive Index Matching: Discard the reagent from the container and replace it with ionic liquid matching reagent IL5-C. Invert to mix immediately after adding. Incubate on a shaker at room temperature for 2-4 hours; additionally, inverting and manually mixing several times during incubation is recommended. Subsequently, discard the reagent and replace it with ionic liquid matching reagent IL2.5-C. Immediately after addition, invert the container to mix thoroughly. Incubate on a shaker at room temperature for 4-6 hours, with additional manual inversions recommended during processing, until the tissue achieves complete transparency.

Precautions

- This kit is intended solely for the optical clearing of ex vivo tissues and must not be used on in vivo tissues.
- This kit has a one-year shelf life; the manufacturing date can be found on the product label.
- All reagents stored at low temperatures must be equilibrated to room temperature before unsealing and use. Reagents may be stored at room temperature if used within one week.
- Upon arrival, it is recommended to aliquot and store the antibody crosslinker. This reagent is prone to degradation upon exposure to moisture. After dilution with PBS, use immediately. The diluted solution will be severely compromised if stored at room temperature for more than 2 hours.
- IL2.5-C and IL5-C may crystallize when stored at 4°C or -20°C. Storage at -80°C prevents crystallization of these reagents.
- If crystallization occurs in IL5-C or IL2.5-C, tightly seal the reagent vessel and incubate in a 50°C water bath or metal bath until crystals fully dissolve (approximately 30 minutes). The reagent can be used normally after complete dissolution.
- Processing duration for each step may be extended based on experimental outcomes. Generally, increase all step durations for thicker tissues or samples subjected to extended fixation.
- To ensure optimal tissue clearing quality, use an orbital shaker or intermittent manual mixing after tissue immersion in reagents.
- Recommended reagent volume: ~3 mL per tissue sample (1 cm^2 × 1 mm thickness). Insufficient volumes may compromise clearing efficacy.
- VIVIT-processed tissues may be stored long-term at -80°C.
- Pausing points may be set at any step except during RIL-C rinsing. Extending other steps (excluding RIL-C rinsing) has minimal impact on results. Overnight processing is recommended for delipidation and IL2.5-C refractive index matching steps.
- DIDC-C effectively removes oil-based pen marks. Always close lids tightly during use to prevent leakage.
- This product is restricted to scientific research by qualified professionals only. Not for clinical diagnosis or treatment.
- For your safety and health, please wear disposable gloves and a mask when using this reagent.
- Keep all reagents away from fire and heat sources, and store them in a cool, dry place below 25°C, avoiding direct sunlight.
- All waste liquids from tissue processing should be collected separately in dedicated containers. Do not mix them with strong oxidizing agents, and avoid pouring waste liquids directly into drains.
- First-time users: Follow the specified volumes/times in the manual and purchase optional reagents as needed. Users with special requirements should contact technical support via our official website.

效果示意 Effect Illustration



参考文献 Reference

VIVIT: Resolving trans-scale volumetric biological architectures via ionic glassy tissue
https://doi.org/10.1016/j.cell.2025.07.023