Solving Long-Standing Challenges! Pioneering New Frontiers in ERβ Research with the Innovative PPZ0506 Antibody

～ Case Study Interview: Perseus Proteomics PPZ0506 Antibody ～

Professor Hirotaka Ishii,
Department of Anatomy and Neurobiology, Graduate School of Medicine, Nippon Medical School



The PPZ0506 antibody provided by Perseus Proteomics, with its outstanding specificity and reliability, has shed new light on the research of Professor Hirotaka Ishii (hereinafter referred to as Professor Ishii), who has been engaged in estrogen receptor beta (ERβ) research for many years, leading to innovative findings. What are the immunohistochemical staining methods established through the introduction of the PPZ0506 antibody, overcoming past difficulties, and what new truths about ERβ expression have emerged from them? We interviewed Professor Ishii about case studies of the PPZ0506 antibody's utilization and the future of his research.

Interviewer (Perseus Proteomics employee): Thank you for taking time out of your busy schedule today. First, could you tell us about your encounter with the PPZ0506 antibody and how it came to be used in your current research?

Professor Ishii: Actually, I have a very long relationship with Perseus. Approximately 25 years ago, when I was a graduate student, I was searching for an ERβ antibody, but there were no good ones available at the time, and I had the bitter experience of a collaborative research project targeting ERβ being suspended. At that time, I discussed an issue for ERβ antibody with Perseus Proteomics, which had just started producing antibodies and selling antibody reagents.

Time passed, and I came across a paper published in Nature Communications. That paper reported that among the ERβ antibodies sold as research reagents worldwide, Perseus's PPZ0506 antibody possessed the sole specificity for human ERβ among the 13 commercially available antibodies[*1]. Seeing this, I decided to try again, thinking, "Although it didn't work previously, with this antibody, I might be able to challenge it once more."

Interviewer: It truly was a fateful reunion. In establishing the immunohistochemical staining method for ERβ using the PPZ0506 antibody, were there any innovations or difficulties you encountered?

Professor Ishii: Yes, there were several important points. We thoroughly optimized several conditions, especially based on our early failure experiences[*2].

First, **antigen retrieval**. One significant factor that previously hindered antibody functionality was insufficient antigen retrieval. In our laboratory, we found heat-induced retrieval to be most effective and optimized the conditions to 10-15 minutes heating process using an autoclave. We also attempted retrieval using a microwave oven, but it resulted in non-specific staining, making it not the optimal choice.

Next, **antibody dilution optimization**. Performing titration at an appropriate concentration is crucial for obtaining specific signals. While human tissues often use dilutions ranging from several hundred-fold to 1500-fold, we found that a 4000-fold dilution was optimal for rat tissues.

Furthermore, **suppressing background in mouse tissues** was also a challenge. Since the PPZ0506 antibody is a mouse monoclonal antibody, when applied to mouse tissues, non-specific background caused by endogenous IgG can be an issue. To address this, it was important to thoroughly perform transcardial perfusion/fixation and adequately remove blood. Additionally, we found that using a commercially available "mouse-on-mouse" blocking agent effectively suppressed background. Surprisingly, in some tissues, specific staining was observed even without blocking.



Finally, **the choice of detection system**. Initially, we used the LSAB method, but endogenous biotin caused background issues. Therefore, we switched to the HRP polymer method, which offers an excellent signal-to-noise ratio (S/N ratio), and obtained very clear staining images. This was a highly effective improvement, especially for detecting molecules with low expression levels like ERβ.

Interviewer: Through these detailed optimizations, you were able to maximize the potential of the PPZ0506 antibody. What new insights regarding ERβ have you gained from this?

Professor Ishii: The most significant findings obtained from research using the PPZ0506 antibody are that **ERβ expression is "highly localized"** compared to previous general perceptions and that there are **"clear species differences."**

Previous research using non-specific antibodies suggested that ERβ was expressed in a wide range of organs, but our results showed that its expression is limited to very specific organs and cells.

For example, we confirmed at the protein level that the expression profile varies significantly among species, such as in the testes in humans, the ovaries and specific regions of the prostate in rats, and the ovaries in mice.

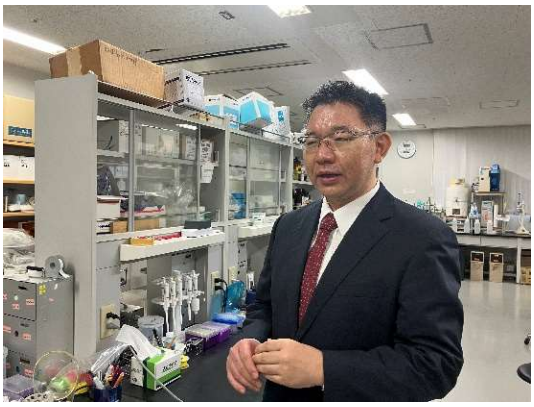
These discoveries correct misunderstandings regarding ERβ expression patterns caused by previous non-specific antibodies and align with mRNA expression data, leading to a more reliable elucidation of ERβ function.

Interviewer: We are very excited about the potential of your research to rewrite the conventional wisdom of ERβ research. Could you please tell us about your expectations for the PPZ0506 antibody in future ERβ research and the prospects for your laboratory?

Professor Ishii: Going forward, we plan to focus particularly on elucidating **the role of ERβ in neuroendocrinology of the brain**. By using the PPZ0506 antibody, we expect to be able to analyze in detail the expression of ERβ and its physiological significance in important neurons involved in hormone secretion, such as specific sex-dimorphic nuclear regions, kisspeptin neurons, dopamine neurons, and vasopressin neurons.

Furthermore, as we belong to the Department of Anatomy within the medical school, **we are also advancing research using human samples**. With ethical committee approval, we believe that by feeding back findings obtained from rats and mice to the human brain and comparatively analyzing ERβ expression patterns and functions across species, and we can contribute to a deeper understanding of higher-order life phenomena.

Interviewer: Thank you very much for your very interesting insights. Finally, could you tell us about the criteria you typically use when selecting antibody reagents?



Professor Ishii: What we value most when choosing antibodies is **"track record and reliability."** Using a new antibody is high-risk because it requires a lot of time and effort for condition optimization. Therefore, we prioritize trying antibodies that have a substantial publication record in papers or have a good reputation from other laboratories.

Among commercially available antibodies, it is not uncommon for those advertised as "specificity guaranteed" to not yield expected results in actual use. Especially when investigating targets with low expression levels or expression in organs that have not been widely studied, the true

specificity of the antibody is called into question. Therefore, we constantly strive to select antibodies that we are confident are truly "highly specific," referring to word-of-mouth and past usage records. The PPZ0506 antibody precisely meets such criteria and is indispensable for future research.

Summary

Through the interview with Professor Ishii, it became clear that the PPZ0506 antibody from Perseus Proteomics has resolved long-standing issues in ERβ immunohistochemical staining and brought new insights into its expression patterns. By combining diligent efforts such as optimizing antigen retrieval, appropriate antibody dilution, and selecting specific detection systems with the excellent specificity of the PPZ0506 antibody, ERβ research is entering a new phase.

Moving forward, Professor Ishii's laboratory reportedly plans to expand its research scope, utilizing the PPZ0506 antibody to investigate the role of ERβ in neuroscience and its expression analysis in the human brain. Perseus Proteomics will continue to provide highly reliable antibody reagents that contribute to solving researchers' challenges and advancing life sciences.

***1:** Andersson, S., Sundberg, M., Pristovsek, N. et al. Insufficient antibody validation challenges oestrogen receptor beta research. *Nat Commun* **8**, 15840 (2017)

***2:** Supplementary material - The optimized immunohistochemical staining protocol for rat and mouse sections using the PPZ0506 antibody developed by Professor Ishii is provided below.

NOTE1: Professor Hirotaka Ishii, Department of Anatomy and Neurobiology, Graduate School of Medicine, Nippon Medical School
https://www.nms.ac.jp/college/pickup_contents/pickup25_ishi.html (Japanese)

NOTE2: Perseus Proteomics Inc. List of antibody products
https://www.ppmx.com/en/business/products/antibody.html

Supplementary Material

Optimized protocols for immunohistochemical staining of rat and mouse sections using PPZ0506 antibody, with some modifications

Tissue preparation

1. Euthanize animals by intraperitoneal injection of mixed anesthesia (medetomidine, midazolam, and butorphanol).
2. Perfuse transcardially with 0.9% (w/v) saline and 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (PB; pH, 7.4).
3. Post-fix organs with the same fixative overnight at 4°C.

Preparation of paraffin-embedded sections of peripheral organs

1. Immerse fixed organs in PB and dehydrate in an ethanol series (70%, 80%, 90%, 95%, 99%, and 100%) and xylene.
2. Embed the fixed organs in paraffin.
3. Slice paraffin-embedded organs at 5-μm thickness on a microtome (RM2235; Leica Biosystems, Nußloch, Germany).
4. Mount sections on Frontier-coated glass slides (Matsunami Glass, Osaka, Japan).
5. Deparaffinize with xylene and hydrate in an ethanol series (100%, 99%, 95%, 90%, 80%, and 70%).
6. Rinse with distilled water (DW) for 5 min.

Preparation of frozen sections of brains

1. Imm$_2$erse fixed organs in 0.1 M PB containing 20% (w/v) sucrose for 2–3 days at 4°C.
2. Freeze the fixed organs in n-hexane at -80°C.
3. Slice frozen organs at a thickness of 30 μm (for mouse brain) or 40 μm (for rat brain) on a cryostat (CM3050S; Leica Biosystems).
4. Rinse three times with 0.1 M phosphate-buffered saline (PBS; pH, 7.4) for 5 min each time.
5. Mount sections on Frontier-coated glass slides.
6. Dry the slides on a slide warmer (PS-53; Sakura Finetek Japan, Tokyo, Japan) at 60°C for 1 h.
7. Rinse with DW for 5 min.

Immunohistochemical staining of rat and mouse sections with PPZ0506

1. Place sections mounted on glass slides in methanol containing 0.3% (v/v) H_2O for 5 min.
2. Rinse with DW and PBS for 5 min each.

3. Autoclave at 121°C for 10 min in citrate-based antigen unmasking solution (H 3300; Vector Laboratories, Newark, CA, USA) for antigen retrieval.
4. Rinse three times with PBS (5 min for each rinse).
5. Blocking Rat: Block with 5% normal goat serum (NGS) in 0.1 M PBS containing 0.1% Triton X-100 (PBST; pH, 7.4) for 30 min. Mouse: Block with blocking reagent (component of Mouse-on-Mouse Polymer IHC Kit; ab269452; Abcam, Cambridge, UK) for 30 min.
6. Primary antibody reaction* Rat: React with PPZ0506 antibody (1:2000 for brain tissues, 1:4000 for peripheral tissues; Perseus Proteomics, Tokyo, Japan) in PBST containing 5% NGS at 4°C overnight. Mouse: React with PPZ0506 antibody (1:2000 for brain tissues, 1:4000 for peripheral tissues) in PBST at 4°C overnight. *To obtain negative controls, prepare sections subjected to solvent without primary antibody.
7. Rinse three times with PBST (5 min for each rinse).
8. Secondary antibody reaction Rat: React with 50% (v/v) goat anti-mouse immunoglobulin G conjugated to a horseradish peroxidase (HRP)-labeled polymer (ab214879; Abcam) in PBST for 2 h. Mouse: React with HRP polymer detector reagent (component of Mouse-on Mouse Polymer IHC Kit; ab269452; Abcam) for 15 min.
9. Rinse three times with PBS (5 min for each rinse).
10. Rinse with 0.05 M Tris-HCl (pH, 7.5) for 5 min.
11. Visualize immunoreactive signals with 200 μg/mL of 3,3′-diaminobenzidine tetrahydrochloride (Merck KGaA, Darmstadt, Germany) and 0.01% (v/v) H_2O_2 in 0.05 M Tris-HCl (pH, 7.5).
12. Counterstain with hematoxylin for peripheral tissues.
13. Rinse with DW, dehydrate in ethanol series (70%, 80%, 90%, 95%, 99%, and 100%), and clear in xylene.
14. Coverslip with Permount Mounting Medium (Thermo Fisher Scientific, Waltham, MA, USA)