



CanAg NSE EIA

**For Research Use Only.
Not for use in
diagnostic procedures.**

REF 420-85

Instructions for use. 2006-11

Enzyme immunometric assay kit
For 96 determinations

GB EXPLANATION OF SYMBOLS
DE BEDEUTUNG DER SYMBOLE
ES EXPLICACIÓN DE SÍMBOLOS
IT SIGNIFICATO DEI SIMBOLI
FR EXPLICATION DES SYMBOLES
DK SYMBOLFORKLARING
GR ΕΠΕΞΗΓΗΣΗ ΤΩΝ ΣΥΜΒΟΛΩΝ
SE SYMBOLFÖRKLARING



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Fecha de caducidad/
Utilizzare entro/Utiliser jusque/
Holdbar til/Hμερομηνία λήξης/
Bäst före datum

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Codigo de lote/
Codice del lotto/Code du lot/
Lotnummer/Αριθμός Παρτίδας/
Lotnummer



Date of manufacture/
Herstellungsdatum/
Fecha de fabricación/
Data di fabbricazione/
Date de fabrication/
Produktionsdato/
Ημερομηνία Παραγωγής/
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Catalogue number/Bestellnummer/
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Référence du catalogue/
Katalognummer/
Αριθμός καταλόγου/
Produktnummer



Manufacturer/Hersteller/Fabri-
cante/Fabbricante/Fabricant/
Producent/τασκευαστής/
Tilverkare



Contains sufficient for <96> tests/
Ausreichend für "96" Ansätze/
Contenido suficiente
para <96> ensayos/
Contenuto sufficiente per "96" saggi/
Contenu suffisant pour "96" tests/
Indeholder tilstrækkeligt
til "96" test/
Περιεχόμενο επαρκές
για «96» εξετάσεις/
Innehåller tillräckligt till "96" tester



Temperature limitation/
Zulässiger Temperaturbereich/
Limite de temperatura/
Limiti di temperatura/
Limites de température/
Temperaturbegrensning/
Περιορισμοί θερμοκρασίας/
Temperaturgräns



Consult Instructions for Use/
Gebrauchsanweisung beachten/
Consulte las instrucciones de uso/
Consultare le istruzioni per l'uso/
Consulter les instructions d'utilisation/
Se brugsanvisning/
Συμβουλευτείτε τις οδηγίες
χρήσης/
Se bruksanvisning



Biological risks/Biogefährdung/
Riesgo biológico/Rischio biologico/
Risques biologiques/
Biologisk fare/Bιολογικοί κίνδυνοι/
Biologisk risk



Contents of kit/Inhalt/Contenido/
Contenido/Contenu/Inndhold/
ανιδραστήρια/Kit innehåll



From mouse/der Maus/de ratón/
Murino/De souris/Mus/απτο ποντίκι/
Från mus



Human/Human/Humano/
Origine Humana/Humaine/Human
δείγματα αναφοράς/Human

INTENDED USE

The CanAg NSE EIA kit is intended for the quantitative determination of NSE in human serum.

SUMMARY AND EXPLANATION OF THE ASSAY

The glycolytic enzyme enolase (2-phospho-D-glycerate hydrolase, EC 4.2.1.11) exists as several dimeric isoenzymes ($\alpha\alpha$, $\alpha\beta$, $\alpha\gamma$, $\beta\beta$ and $\gamma\gamma$) composed of three distinct subunits α , β and γ . The γ unit is found either in a homologous $\gamma\gamma$ - or in a heterologous $\alpha\gamma$ -isoenzyme and is known as neuron-specific enolase (NSE). The monoclonal antibodies used in the CanAg NSE EIA bind to the γ -subunit of the enzyme and thereby detects both the $\gamma\gamma$ and the $\alpha\gamma$ forms (1, 2).

PRINCIPLE OF THE TEST

The CanAg NSE EIA is a solid phase, non-competitive immunoassay based on two monoclonal antibodies (derived from mice) directed against two separate antigenic determinants of the NSE molecule. The monoclonal antibodies (MAb) used bind to the γ -subunit of the enzyme and thereby detects both the $\gamma\gamma$ and the $\alpha\gamma$ form. Calibrators and samples are incubated together with biotinylated Anti-NSE MAb E21 and horseradish peroxidase (HRP) labelled Anti-NSE MAb E17 in streptavidin coated microstrips. After washing, buffered Substrate/Chromogen reagent (hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine) is added to each well and the enzyme reaction is allowed to proceed. During the enzyme reaction a blue colour will develop if antigen is present. The intensity of the colour development is proportional to the amount of NSE present in the samples. The colour intensity is determined in a microplate spectrophotometer at 620 nm (or optionally at 405 nm after addition of Stop Solution).

Calibration curves are constructed for each assay by plotting absorbance value versus the concentration for each calibrator. The NSE concentrations of samples are then read from the calibration curve.

REAGENTS

- Each CanAg NSE EIA kit contains reagents for 96 tests.
- The expiry date of the kit is stated on the label on the outside of the kit box.
- Do not use the kit beyond the expiry date.
- Do not mix reagents from different kit lots.
- Store the kit at 2–8°C. Do not freeze.
- Opened reagents are stable according to the table below provided they are not contaminated, stored in resealed original containers and handled as prescribed. Return to 2–8°C immediately after use.

Component	Quantity	Storage and stability after first opening
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MICROPLA

Streptavidin Microplate	1 Plate	2–8°C until expiry date stated on the plate
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12 x 8 breakable wells coated with streptavidin. After opening, immediately return unused strips to the aluminium pouch containing desiccant and reseal carefully to keep dry.

Component	Quantity	Storage and stability after first opening			
NSE Calibrators	10 vials, lyophilised	2 weeks at 2–8°C 3 months at –20°C			
<table border="1"><tr><td>CAL</td><td>NSE</td><td>A</td></tr></table>	CAL	NSE	A	2 x 0.75 mL	
CAL	NSE	A			
<table border="1"><tr><td>CAL</td><td>NSE</td><td>B</td></tr></table>	CAL	NSE	B	2 x 0.75 mL	
CAL	NSE	B			
<table border="1"><tr><td>CAL</td><td>NSE</td><td>C</td></tr></table>	CAL	NSE	C	2 x 0.75 mL	
CAL	NSE	C			
<table border="1"><tr><td>CAL</td><td>NSE</td><td>D</td></tr></table>	CAL	NSE	D	2 x 0.75 mL	
CAL	NSE	D			
<table border="1"><tr><td>CAL</td><td>NSE</td><td>E</td></tr></table>	CAL	NSE	E	2 x 0.75 mL	
CAL	NSE	E			

The lyophilised calibrators contain human NSE in a protein matrix with 0.01 % methyl-isothiazolone (MIT) as preservative. To be reconstituted with water before use.

NOTE: The exact NSE concentration is lot specific and is indicated on the label of each vial.

BIOTIN	Anti-NSE
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Biotin Anti-NSE	1 x 15 mL	2–8°C until expiry date stated on the vial
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Biotin Anti-NSE monoclonal antibody from mouse, approximately 2 µg/mL. Contains phosphate buffer (pH 7.1), bovine serum albumin, blocking agents, an inert blue dye and 0.01 % methyl-isothiazolone (MIT) as preservative. To be mixed with Tracer, HRP Anti-NSE before use.

CONJ	Anti-NSE
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Tracer, HRP Anti-NSE	1 x 0.75 mL	2–8°C until expiry date stated on the vial
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Stock solution of HRP Anti-NSE monoclonal antibody from mouse, approximately 40 µg/mL. To be mixed with Biotin Anti-NSE prior to use. Contains 0.02 % methyl-isothiazolone (MIT), 0.02 % bromonitrodioxane and 20 ppm Proclin™ 300 as preservatives.

Component	Quantity	Storage and stability after first opening
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SUBS	TMB
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TMB HRP-Substrate	1 x 12 mL	2–8°C until expiry date stated on the vial
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Ready for use. Contains buffered hydrogen peroxide and 3, 3', 5, 5' tetramethylbenzidine (TMB).

STOP

Stop Solution	1 x 15 mL	2–8°C until expiry date stated on the vial
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Ready for use. Contains 0.12 M hydrochloric acid.

WASHBUF	25X
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Wash Concentrate	1 x 50 mL	2–8°C until expiry date stated on the bottle
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To be diluted with water 25 times before use. A Tris-HCl buffered salt solution with Tween 20. Contains Germall II as preservative.

Indications of instability

The TMB HRP-Substrate should be colourless or slightly bluish. A blue colour indicates that the reagent has been contaminated and should be discarded.

WARNINGS AND PRECAUTIONS

For Research Use Only. Not for use in diagnostic procedures.

- Please refer to the U.S. Department of Health and Human Services (Bethesda, Md., US) publication No. (CDC) 88-8395 on laboratory safety or any other local or national regulation.
- Handle all serum specimens as potentially infectious.
- Follow local guidelines for disposal of all waste material.

Caution

Each donor unit used in the preparation of human source reagent has been tested and found to be Non Reactive for HIV-1/2 Antibody, HCV Antibody and Hepatitis B Surface Antigen (HBsAg). Since no method can completely rule out the presence of blood borne diseases, the handling and disposal of human source reagents from this product should be made as if they were potentially infectious.

SPECIMEN COLLECTION AND HANDLING

The CanAg NSE EIA is intended for use with serum. Collect blood by venipuncture and separate the serum according to common procedures. Serum should be separated from the clot within 60 minutes of collection to avoid leaking of NSE from blood cells. Do not use haemolysed samples. Plasma is not recommended since significant amounts of NSE can be released from platelets. Samples can be stored at 2–8°C for 24 hours. For longer periods store samples at -70°C or below. Samples should not be stored in a self-defrosting freezer and not be thawed and refrozen before analysis. Allow frozen samples to thaw slowly at 2–8°C over night and then bring the samples to room temperature before analysis.

PROCEDURE

Materials required but not supplied with the kit

1. Microplate shaker

Shaking should be medium to vigorous. Longitudinal shaking approximately 200 strokes/min, oscillations 700–900/min.

2. Microplate wash device

Automatic platewash capable of performing 1 and 6 washing cycles, or semi manual microplate washing device connected to vacuum pump or water-jet vacuum and a liquid trap for retaining aspirated liquid.

The Nunc Immuno-8 manual strip washer is recommended if an automatic microplate wash is not used.

3. Microplate spectrophotometer

With a wavelength of 620 nm and/or 405 nm, and an absorbance range of 0 to 3.0.

4. Precision pipettes

With disposable plastic tips for dispensing microlitre volumes. An 8-channel pipette or respenser pipette with disposable plastic tips for delivery of 100 µL is useful but not essential. Pipettes for dispensing millilitre volumes.

5. Distilled or deionized water

For reconstitution of NSE Calibrators and for preparation of diluted wash solution.

Procedural notes

1. A thorough understanding of this package insert is necessary to ensure proper use of the CanAg NSE EIA kit. The reagents supplied with the kit are intended for use as an integral unit. Do not mix identical reagents from kits having different lot numbers. Do not use the kit reagents after the expiry date printed on the outside of the kit box.
2. Reagents should be allowed to reach room temperature (20–25°C) prior to use. The assay should only be performed at temperatures between 20–25°C to obtain accurate results. Frozen sera must be gently but thoroughly mixed after thawing.
3. Before starting to pipette calibrators and specimens it is advisable to mark the strips to be able to clearly identify the samples during and after the assay.
4. A careful washing procedure of the strips is essential. Ensure that each well is filled up completely to the top edge, that the aspiration of the wells between and after the washing cycles is complete and that the wells are empty. If there is moisture left, invert the plate and tap it carefully against absorbent paper.

Automatic strip washer: Follow the manufacturer's instructions for maintenance and wash the required number of wash cycles prior to and after each incubation step.

The aspiration/wash device should not be left standing with the wash solution for long periods, as the needles may get clogged giving poor liquid delivery and suction.

5. The TMB HRP-Substrate is very sensitive for contamination. For optimal stability of the TMB HRP-Substrate, pour the required amount from the vial to a carefully cleaned reservoir or preferably a disposable plastic tray to avoid contamination of the reagent. Be sure to use clean disposable plastic pipette tips (or respenser pipette tip).
6. Be sure to use clean disposable plastic pipette tips and a proper pipetting technique when handling samples and reagents. Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid. A proper pipetting technique is of particular importance when handling the TMB HRP-Substrate solution.

Protocol Sheet

CanAg NSE EIA REF 420-85

Mix the components directly before use. Use shaking conditions according to the Instructions.

Step	Bottle/Plate		Procedure																					
	CAL	NSE																						
1. Prepare NSE Calibrators	A, B, C, D, E		Add 0.75 mL of distilled water to each vial and mix gently. Allow to stand for at least 15 minutes. NOTE: The exact concentration of each calibrator is stated on the label. This value of the calibrators should be used for calculations.																					
2. Prepare Wash Solution	WASHBUF	25X	Dilute 50 mL of Wash Concentrate with 1 200 mL of distilled water or deionized water.																					
3. Prepare Antibody Solution	CONJ	Anti-NSE	Mix 50 µL of Tracer, HRP Anti-NSE with 1 mL of Biotin Anti-NSE per strip:																					
	BIOTIN	Anti-NSE																						
			<table border="1"><thead><tr><th>No. of Strips</th><th>HRP Anti-NSE (µL)</th><th>Biotin Anti-NSE (mL)</th></tr></thead><tbody><tr><td>1</td><td>50</td><td>1</td></tr><tr><td>2</td><td>100</td><td>2</td></tr><tr><td>3</td><td>150</td><td>3</td></tr><tr><td>4</td><td>200</td><td>4</td></tr><tr><td>5</td><td>250</td><td>5</td></tr><tr><td>6</td><td>300</td><td>6</td></tr></tbody></table>	No. of Strips	HRP Anti-NSE (µL)	Biotin Anti-NSE (mL)	1	50	1	2	100	2	3	150	3	4	200	4	5	250	5	6	300	6
No. of Strips	HRP Anti-NSE (µL)	Biotin Anti-NSE (mL)																						
1	50	1																						
2	100	2																						
3	150	3																						
4	200	4																						
5	250	5																						
6	300	6																						

7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12
4. Wash	MICROPLA	Wash each well once with Wash Solution
5. Add calibrators and samples	CAL NSE A, B, C, D, E	25 µL in each well
6. Add Antibody Solution	ANTIBODY SOLUTION	100 µL in each well
7. Incubate	MICROPLA	1 hour shaking at room temperature
8. Wash	MICROPLA	Wash each well six times with Wash Solution
9. Add TMB HRP-Substrate	SUBS TMB	100 µL in each well
10. Incubate	MICROPLA	30 min shaking at room temperature
11. Read absorbance	MICROPLA	620 nm
Alt.11 Add Stop Solution	STOP	100 µL in each well
Alt.12 Incubate	MICROPLA	1 min shaking at room temperature
Alt.13 Read absorbance	MICROPLA	Read at 405 nm within 15 min

Preparation of reagents	Stability of prepared reagent
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NSE Calibrators	2 weeks at 2–8°C
	3 months at -20°C

Add exactly 0.75 mL of distilled water to each vial and mix gently. Allow standing for at least 15 minutes to reconstitute. **NOTE:** The concentration of the calibrators is stated on the labels and should be used for calculation of the results.

Wash Solution	2 weeks at 2–25°C in a sealed container
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Pour the 50 mL Wash Concentrate into a clean container and dilute 25-fold by adding 1200 mL of distilled or deionised water to give a buffered Wash Solution.

Antibody Solution	3 weeks at 2–8°C
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Prepare the required quantity of Antibody Solution by mixing 50 µL of Tracer, HRP Anti-NSE with 1 mL of Biotin Anti-NSE per strip (see table below):

No. of Strips	Tracer, HRP Anti-NSE (µL)	Biotin Anti-NSE (mL)
1	50	1
2	100	2
3	150	3
4	200	4
5	250	5
6	300	6
7	350	7
8	400	8
9	450	9
10	500	10
11	550	11
12	600	12

Be sure to use a clean plastic or glass bottle for preparation of Antibody Solution. **Alternative:** Pour the content of the Tracer, HRP Anti-NSE into the vial of Biotin Anti-NSE and mix gently. Be sure that all content of the Tracer is transferred to the vial of Biotin Anti-NSE.

NOTE: The Antibody Solution is stable for 3 weeks at 2–8°C. Do not prepare more Antibody Solution than will be used within this period and make sure that it is stored properly.

Assay procedure

Perform each determination in duplicate for both calibrators and unknown samples. A calibration curve should be run with each assay. All reagents and samples must be brought to room temperature (20–25 °C) before use.

1. Start to prepare NSE Calibrators, Wash Solution and Antibody Solution. It is important to use clean containers. Follow the instructions carefully.
2. Transfer the required number of microplate strips to a strip frame. (Immediately return the remaining strips to the aluminium pouch containing a desiccant and reseal carefully). Wash each strip once with the Wash Solution. Do not wash more strips than can be handled within 30 min.
3. Pipette 25 μL of the NSE Calibrators (CAL A, B, C, D, E) and unknown specimens (unknowns-Unk) into the strip wells according to the following scheme:

	1	2	3	4	5	6	7 etc
A	Cal A	Cal E	4th Unk				
B	Cal A	Cal E	etc				
C	Cal B	1st Unk					
D	Cal B	1st Unk					
E	Cal C	2nd Unk					
F	Cal C	2nd Unk					
G	Cal D	3rd Unk					
H	Cal D	3rd Unk					

4. Add 100 μL of Antibody Solution to each well using a 100 μL precision pipette (or an 8-channel 100 μL precision pipette). Avoid carry-over by holding the pipette tip slightly above the top of the well and avoid touching the plastic strip or surface of the liquid.
5. Incubate the plate for 1 hour (± 10 min) at room temperature (20-25°C) with constant shaking of the plate using a microplate shaker.
6. After the incubation aspirate and wash each strip 6 times.

7. Add 100 μL of TMB HRP-Substrate to each well using the same procedure as in item 4. The TMB HRP-Substrate should be added to the wells as quickly as possible and the time between addition to the first and last well should not exceed 5 min.
8. Incubate for 30 min (\pm 5 min) at room temperature with constant shaking. Avoid exposure to direct sunlight.
9. Immediately read the absorbance at 620 nm in a microplate spectrophotometer.

Option

If the laboratory does not have access to a microplate spectrophotometer capable of reading at 620 nm the absorbance can be determined as in item 10.

10. Add 100 μL of Stop Solution, mix and read the absorbance at 405 nm in a microplate spectrophotometer within 15 min after addition of Stop Solution.

Measurement range

The CanAg NSE EIA measures concentrations between 1 and approximately 150 $\mu\text{g/L}$. If NSE concentrations above the measuring range are to be expected, it is recommended to dilute samples with normal human serum prior to analysis.

NOTE: The serum used for dilution should also be measured in order to determine the endogenous NSE concentration (see "Calculation of results").

Reference materials

Since no common reference material is available for NSE antigen, CanAg NSE EIA Calibrator values are assigned against a set of in-house reference standards.

CALCULATION OF RESULTS

If a microplate spectrophotometer with built-in data calculation program is used refer to the manual for the spectrophotometer and create a program using the concentration stated on the label of each of the NSE calibrators.

For automatic calculation of NSE results it is recommended to use either of the following methods:

- Cubic spline curve fit method. Calibrator A should be included in the curve with the value 0 $\mu\text{g/L}$.
- Spline smoothed curve fit method. Calibrator A should be used as plate blank.
- Interpolation with point-to-point evaluation. Calibrator A should be included in the curve with the value 0 $\mu\text{g/L}$.

- Quadratic curve fit method. Calibrator A should be included in the curve with the value 0 µg/L.

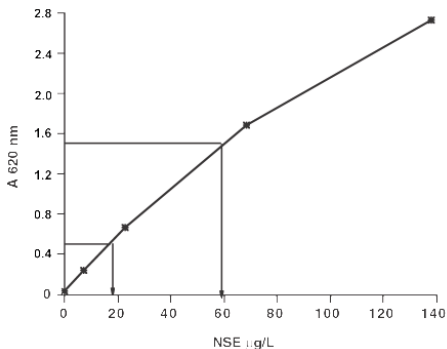
NOTE: 4-Parametric or Linear regression evaluation methods should not be used.

For manual evaluation, a calibration curve is constructed by plotting the absorbance (A) values obtained for each NSE Calibrator against the corresponding NSE concentration (in µg/L), see figure. The unknown NSE concentrations can then be read from the calibration curve using the mean absorbance value of each specimen. If samples in an initial analysis give NSE levels above the concentration of calibrator E, it is necessary to dilute the sample 1/10 with normal human serum in order to obtain accurate results. The result should then be calculated according to the following procedure:

$$\text{Dilution 1/10: } 10 \times ([\text{NSE}]_{\text{Diluted sample}} - (0.9 \times [\text{NSE}]_{\text{Normal human serum}}))$$

Example of results

Specimen	Calibrator values	Mean abs value (A)	NSE µg/L
CAL NSE A	0 µg/L	0.037	
CAL NSE B	7.5 µg/L	0.238	
CAL NSE C	22.9 µg/L	0.663	
CAL NSE D	68.4 µg/L	1.688	
CAL NSE E	138.0 µg/L	2.720	
Specimen 1		0.518	17.5
Specimen 2		1.474	57.8



Example, do not use this curve to determine assay results.

The exact NSE concentration is indicated on the label of each calibrator vial.

LIMITATIONS OF THE PROCEDURE

Serum should not contain visible haemolysis (the absorbance at 500 nm for non-turbid sample should not exceed 0.3) since erythrocytes contain significant amounts of NSE (3). Prolonged storage of whole blood can cause release of NSE from the blood cells.

Anti-reagent antibodies (human anti-mouse antibody (HAMA) or heterophilic antibodies) in the sample may occasionally interfere with the assay, even though specific blocking agents are included in the buffer.

WARRANTY

The performance data presented here were obtained using the assay procedure indicated. Any change or modification of the procedure not recommended by Fujirebio Diagnostics may affect the results, in which event Fujirebio Diagnostics disclaims all warranties expressed, implied or statutory including the implied warranty of merchantability and fitness for use.

LITERATURE REFERENCES

1. Paus E. and Nustad K., (1989) Immunoradiometric Assay for $\alpha\gamma$ - and $\gamma\gamma$ -Enolase (Neuron-Specific Enolase), with Use of Monoclonal Antibodies and Magnetizable Polymer Particles. *Clin. Chem.* 35: 2034-2038.
2. Dahlén U., Karlsson B., Nilsson O. and Uhl W., (1995) Development of an Enzyme Immunoassay, NSE-Enzymun Test For Determination of Neuron-Specific Enolase. XXIII International Society for Oncodevelopmental Biology and Medicine, Montréal, Québec .
3. Pålman S., Esscher T., Bergvall P. and Odelstad L., (1984) Purification and characterization of human neuron-specific enolase: Radioimmunoassay development. *Tumor Biol.* 5: 127–139.



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