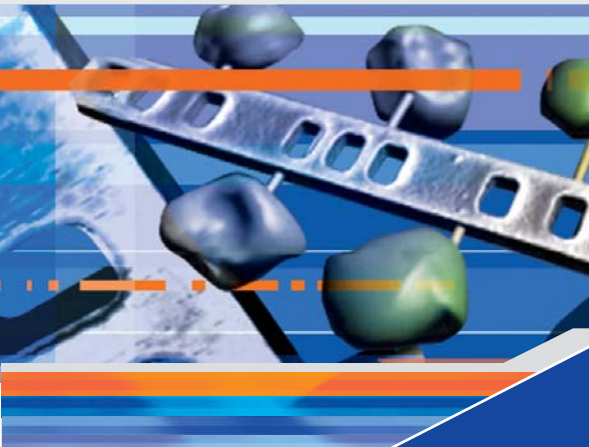




PRONOSTICS  
Knowledge for Health



# UltraPlex™ Coeliac

40 samples 200 TESTS

A multiplexed immunoassay for the semi-quantitative detection of IgA-specific and pan-Ig antibodies to gliadin and tissue transglutaminase and semi-quantitative detection of total serum IgA.

FOR IN VITRO DIAGNOSTIC USE

Pronostics Ltd. is an ISO 13485:2003 certified company

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# UltraPlex™ Coeliac

400 samples (200 tests)

For in vitro diagnostic use only.

Store at 2-8°C upon receipt.

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## Intended use

The UltraPlex™ Coeliac Assay is intended for use in the detection of antibodies to gliadin and tissue transglutaminase and detection of IgA in human serum.

## Summary and explanation of the assay

Coeliac disease is a disorder primarily affecting the gastrointestinal tract that is characterised by chronic inflammation of the mucosa, resulting in atrophy of intestinal villi. Other clinical manifestations may be exhibited anytime during childhood or adult life. Symptoms of coeliac disease include tiredness, nausea, diarrhoea, headaches, skin problems, hair loss, bloating, depression, constipation, weight loss, mouth ulcers, and anaemia. If the condition goes untreated there is a raised risk of infertility, vitamin and mineral deficiencies, osteoporosis, cancer and other gastrointestinal problems. Coeliac disease is also associated with skin disorders such as dermatitis herpetiformis.

Genetic and environmental factors interact in the cause of celiac disease. Certain HLA subtypes have been shown to be more susceptible to the disease (HLA-DQ2 and HLA-DQ8). The main environmental trigger has been identified as gliadin which is present in wheat, rye and barley. There is no cure for coeliac disease, but most patients experience complete remission after exclusion of these grains from their diet.

For a number of years celiac disease was considered rare. However, due to modern advancements in diagnosis, it is now believed that coeliac disease may affect up to 1 in 100 people in the UK - but with 80% of people undiagnosed.

The UltraPlex™ Coeliac assay is a multiplexed immunoassay for the semi-quantitative detection of IgA specific and pan-Ig antibodies to gliadin and tissue transglutaminase, together with semi-quantitative detection of total IgA in human serum in one simple assay. The UltraPlex™ SmartStation performs all liquid handling, incubation, tracking of samples and pipettes the completed assay microparticles into a reading plate. The UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each UltraCode, enabling the five immunoassays to be performed simultaneously on a serum sample in a single well.

## Principle of the UltraPlex™ Coeliac assay

The UltraPlex™ Coeliac system contains pre-formulated SmartBeads (1 per well). Each SmartBead contains two antigens (gliadin and tissue transglutaminase) and an IgA specific antibody, each of which is bound to a distinct UltraCode (barcoded microparticle). Details of the antigens are given in Appendix 1.

In addition to the three UltraCodes with specific coatings, a fourth control UltraCode with no antigen coating (Qmax zero) is also present. This enables the monitoring of non-specific binding of samples to the UltraCodes.

Diluted serum samples are incubated with the SmartBeads to allow any antibodies present in the serum to bind to the antigens. After washing away unbound antibodies, the bound antibodies are detected using an anti-human IgA specific and separately an anti-human pan Ig fluorescent conjugate. After incubation, excess detection antibody is washed away. The UltraPlex™ SmartReader detects the fluorescence associated with each UltraCode and gives a positive or negative result for each individual immunoassay. An indication of the likelihood of each patient sample being deficient in total IgA is also given.

## Reagents and materials supplied

**COELIAC ASSAY PLATE** One 96 well filter bottom microplate containing 1 SmartBead per well and sealed in an aluminium bag with a drying agent. Ready to use.

**SAMPLE DILUTION BUFFER (5x)** One 12 ml bottle of concentrated solution, containing a detergent and protein in phosphate buffered saline with preservative. Dilute 1 in 5 to a final volume of 60 ml.

**WASH BUFFER (10x)** One 30 ml bottle of concentrated solution, containing a detergent in phosphate buffered saline with preservative. Dilute 1 in 10 to a final volume of 300 ml.

**NEGATIVE CONTROL** A single vial containing 600µl diluted normal human serum. The expected results are given on the control certificate. Ready to use.

**IgA AND PAN Ig CONTROLS 1-3** Six vials each containing 300 µl of diluted human serum. The expected results are given on the control certificate. Ready to use.

**IgA-SPECIFIC DETECTION ANTIBODY** One 10 ml bottle of anti-human IgA dye conjugate in protein-stabilising matrix with a preservative. Ready to use.

**PAN-Ig DETECTION ANTIBODY** One 10 ml bottle of anti-human Ig dye conjugate in protein-stabilising matrix with a preservative. Ready to use.

**DECODING BUFFER** One 27 ml bottle of buffer containing glycerol in phosphate buffered saline with a preservative. Ready to use.

**READING PLATE** One 96 well clear bottom black microtitre plate.

**BATCH ID TUBE** One vial displaying the batch record information.

## Storage and stability

Store the kit at 2–8°C in the original container until use. The reagents are stable until the expiration date on the kit. Do not expose kit to heat, sun or strong light during storage or usage. Do not freeze.

## Materials and equipment required

1. Distilled or de-ionised water and clean measuring cylinders for the dilution of buffers.
2. UltraPlex™ SmartReader.
3. UltraPlex™ SmartStation.
4. One 96 deep well plate (Sarstedt Cat# 82.1970.002).
5. Four boxes Rainin 1000 µl tips (Anachem Cat# RT-L1000).
6. Plate sealing tape.

## Warnings and precautions

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1. For research use only.
2. Not for internal use in humans or animals.
3. Not to be used as a standalone product in making diagnosis, monitoring or screening decisions. The result of this test must not be used for determination of medical intervention.
4. The controls contain human source components, which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, hepatitis B virus, or other infectious agents are absent, these reagents should be handled as per local regulations.
5. Optimal results will be obtained by strict adherence to the test protocol.
6. Avoid contact of reagents and samples with the eyes, skin and clothing. Wear disposable gloves, lab clothing and eye protection while handling samples and reagents. Wash hands thoroughly when finished. If contact occurs, wash thoroughly with water or at eyewash stations, and consult a physician.
7. The components of this kit are intended for use as an integral unit. The components of different units should not be mixed.

8. This product contains components preserved with sodium azide. Sodium azide may react with lead and copper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.
9. Do not use kit components past the stated expiry dates. Avoid microbial contamination of all reagents and samples or incorrect results may occur.
10. The procedure is for running 40 tests simultaneously rather than in small batches.

## Specimen collection and handling

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8°C for up to seven days or frozen for up to six months. If frozen, ensure samples are thoroughly defrosted and mixed before use.
3. Avoid repetitive freezing and thawing of serum sample. Lipaemic, haemolysed or contaminated samples may give poor results and should not be tested.
4. A minimum sample volume of 100 µl is required for the UltraPlex™ SmartStation.
5. Up to 40 serum samples can be tested per run.
6. Samples must be contained in tubes compatible with use with the UltraPlex™ SmartStation sample racks.

## Preparation for the assay

1. Bring all specimens and kit reagents to room temperature (18-25°C) and gently mix.
2. Dilute contents of wash buffer concentrate 10x to 300 ml with distilled or de-ionised water in a suitable storage container.
3. Dilute contents of sample dilution buffer concentrate 5x to 60 ml with distilled or de-ionised water in a suitable storage container.
4. Briefly invert to mix and then centrifuge the tubes containing the controls to ensure the liquid is at the bottom of the tubes.
5. Ensure that the samples are fully mixed, especially if they have been stored for a long period of time.
6. It is important to not introduce air bubbles into either the controls or samples during mixing.

## UltraPlex™ Coeliac assay procedure

The assay procedure is composed of two elements. Firstly, the UltraPlex™ SmartStation robotic liquid handling system performs all liquid handling, incubation, tracking of samples and then pipettes the completed assay microparticles into a reading plate. Secondly, the UltraPlex™ SmartReader detects, identifies and measures the fluorescence of each UltraCode and provides the data output.

Separate operating manuals describe the operation of the UltraPlex™ SmartStation and SmartReader, and should be read thoroughly in conjunction with this user guide. Those operating manuals describe in detail the set-up, maintenance and shut-down procedures of the SmartStation and SmartReader.

The aspects of the running of the SmartStation that are specific to the UltraPlex™ Coeliac assay are described below:

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1. The UltraPlex™ Coeliac assay is designed to test 40 samples on a single assay plate. Load these samples into positions 1 – 40 of barcode cassettes 1 – 3 (from top to bottom in cassette 1, then cassettes 2 and 3).
2. Load the controls and standards provided onto the third barcode cassette of the SmartStation in the following order:
  1. Negative control
  2. IgA Positive control 1
  3. IgA Positive control 2
  4. IgA Positive control 3
  5. Pan-Ig positive control 1
  - 6 Pan-Ig positive control 2
  7. Pan-Ig positive control 3
3. Load the batch number tube in position 16 of barcode cassette 3.
4. Load the barcode cassettes onto the SmartStation deck in the order cassette 1 to cassette 3 from left to right.
5. Load the reagent racks as follows:
  1. 30 ml of diluted sample dilution buffer
  2. 30 ml of diluted sample dilution buffer
  3. All of IgA-specific detection antibody (10 ml)
  4. All of provided decoding buffer (27 ml)

The remainder of the set-up of the SmartStation SmartStation is not unique to the UltraPlex™ Coeliac assay, and is detailed in the operating instructions for the SmartStation. For convenience they are also provided in summary form below and in the quick start guide.

1. Place diluted wash buffer in the wash buffer trough.
2. Load four full tip boxes on the deck.
3. Place a fresh piece of blotting paper on the blotting block.
4. Load the deep well dilution plate, read plate and assay plate onto the deck in their respective positions, with well A1 in the top left in each case.

The assay can now be started using the **UltraPlex™ Coeliac** process in the Lirix software, as described in the SmartStation operating manual.

Following completion of the liquid handling steps seal the UltraPlex™ Coeliac reading plate using a plate sealer and transfer plate to the SmartReader system for detection, identification and measurement of the fluorescence of each UltraCode. This procedure is detailed in the SmartReader operating manual.

## Interpretation of the results

The UltraPlex™ SmartDecode software determines whether the samples are negative, borderline or positive for IgA-specific and pan-Ig antibodies to gliadin and tissue transglutaminase.

It also returns a semi-quantitative result for levels of total IgA in the serum sample.

## Quality control

In order for the assay to be valid, all the following criteria must be met:

- Standards and positive and negative controls must be included in each assay run.
- The values obtained for all the controls should be in the ranges specified on the QC certificate

**If any of the above criteria are not met, the assay is invalid and should be repeated.**

## Performance characteristics

### Precision

Intra-assay precision (within an assay) was calculated for each sample from the results of four replicates in a single assay.

Inter-assay precision (between assays) was calculated from the results of samples tested on three different assays with four replicates of each sample on each assay.

The results shown are mean fluorescence intensities following subtraction of the Qmax zero background UltraCode.

| Assay              | CV (%) |
|--------------------|--------|
| IgA vs. gliadin    | 11.5   |
| IgA vs. tTG        | 8.7    |
| Total IgA          | 12.7   |
| Pan Ig vs. gliadin | 13.6   |
| Pan Ig vs. tTG     | 14.8   |

Table 1. Intra-assay precision data

| Antigen            | CV (%) |
|--------------------|--------|
| IgA vs. gliadin    | 11.6   |
| IgA vs. tTG        | 7.9    |
| Total IgA          | 5.4    |
| Pan Ig vs. gliadin | 8.2    |
| Pan Ig vs. tTG     | 13.9   |

Table 2. Inter-assay precision data

## Warranty

The UltraPlex™ Coeliac assay is warranted to perform as described in this packaging insert. Any change or modification to the procedure as described by Pronostics Ltd. may affect the results and is not recommended. Pronostics Ltd. disclaims any implied warranty of merchantability or fitness for a particular use, and in no event will Pronostics Ltd. be liable for consequential or indirect damages.

## References

1. Green P. H., Jabri, B. (2003). Coeliac Disease. *Lancet* **362**: 383-91.
2. Murray, J. A. (1999). The widening spectrum of celiac disease. *Am J Clin Nutrit* **69**: 354-65.
3. Hörnell, A. (2005). Effects of a gluten-free diet on gastrointestinal symptoms in celiac disease. *Am J Clin Nutrit* **81**: 1452-3.

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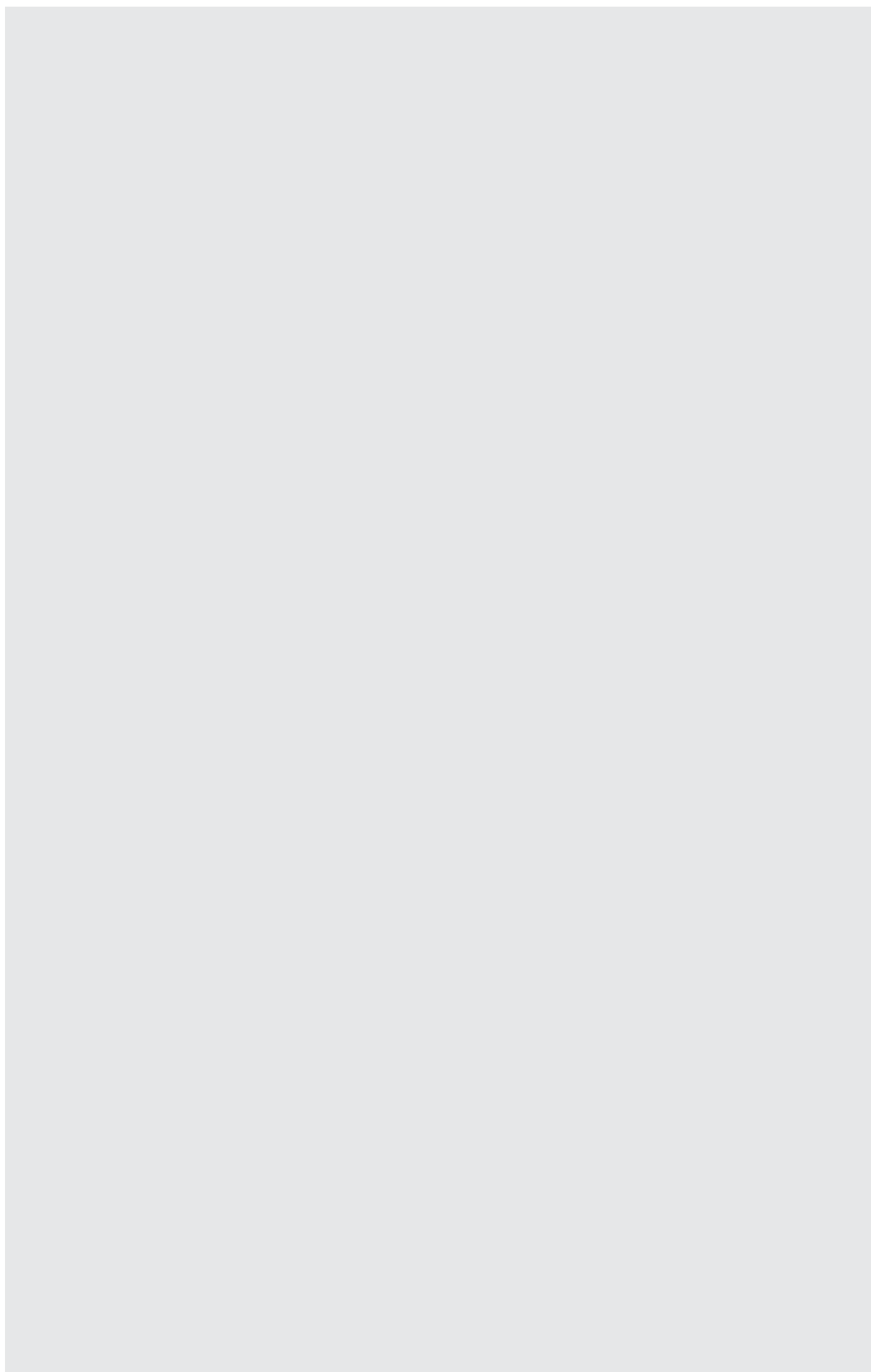
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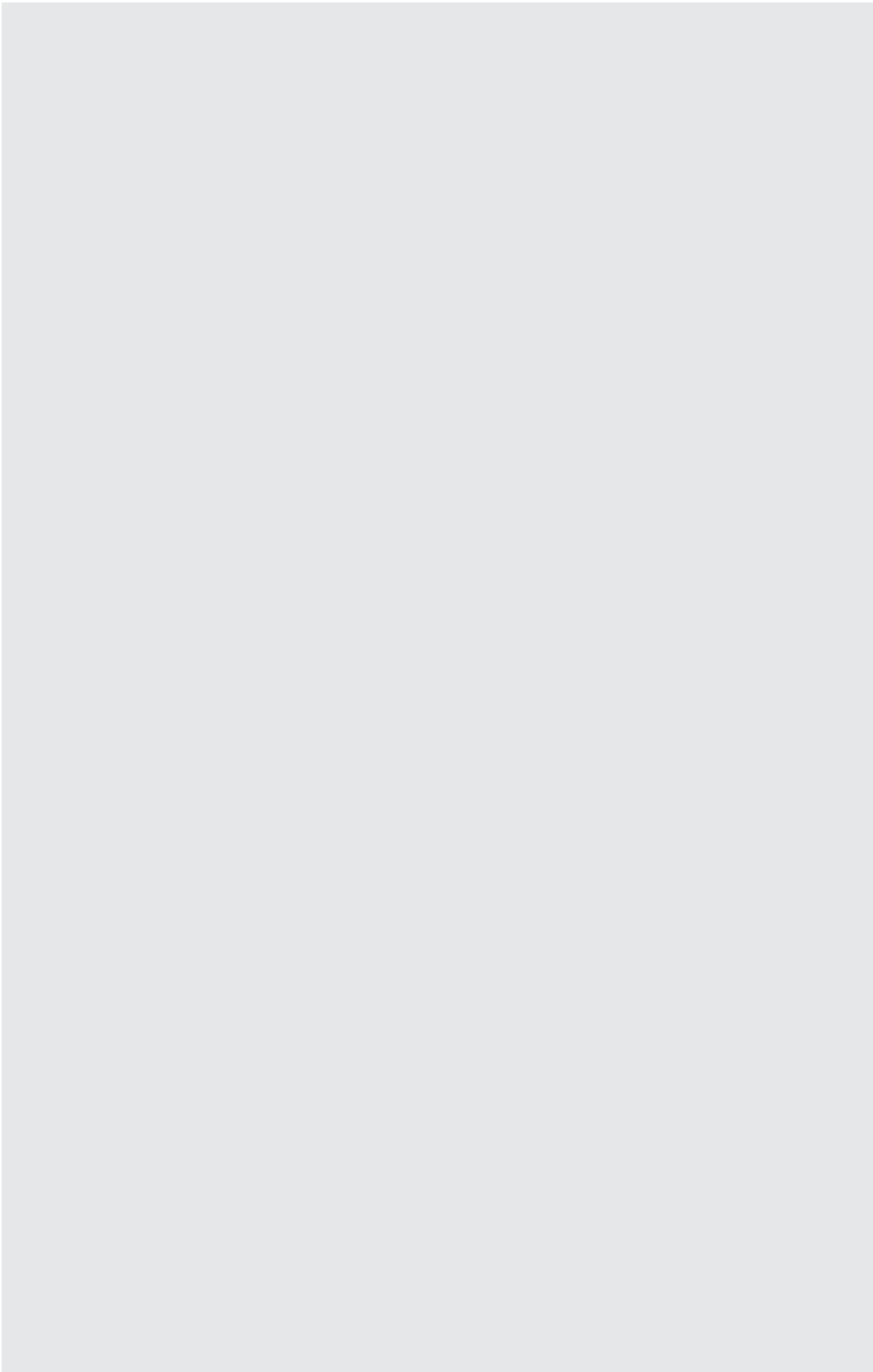
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## Appendix 1. Details of antigens

| Antigen                 | Description  | Purity            |
|-------------------------|--|-------------------|
| Gliadin                 | Crude gliadin from wheat gluten                      | Unknown           |
| Tissue transglutaminase | Recombinant.<br>Expressed in E. coli bacterial cells | >95 % by SDS-PAGE |

## NOTES





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